BANANA WILT

BY
E. W. BRANDES

A DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY, IN THE UNIVERSITY OF MICHIGAN

Reprinted from PHYTOPATHOLOGY, VOL. IX, NO. 9,
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A banana plant of the Gros Michel variety in Costa Rica attacked by the wilt organism.

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E. W. BRANDES

WITH PLATES XXI TO XXXIV AND FIVE FIGURES IN THE TEXT

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[1] "The work on this problem was begun while the writer was connected with the Porto Rico Agricultural Experiment Station at Mayaguez, Porto Rico; it was continued for one and one-half years at the New York State College of Agriculture, Cornell University, and was completed in a final year at the University of Michigan. The writer desires to acknowledge his great indebtedness to Professor H. H. Whetzel, of Cornell, for facilities and advice in conducting the work, and to thank Professor F. C. Newcombe, of the University of Michigan, for the facilities of the laboratory and advice in preparing the dissertation."

I. THE HOST

1. EXTENT OF THE INDUSTRY

The extensive cultivation of bananas (*Musa sapientum*) in the American tropics for exportation to North America and Europe is comparatively recent. A few bunches were imported into this country early in the past century but were regarded only as a horticultural curiosity. No systematic effort had been made towards production in quantities sufficient to create a steady demand or a fixed market price until 1885, when a small fruit company, capitalized at $20,000, was formed in Boston to promote the importation of bananas. The venture proved a success. The numerous obstacles in the way of raising and transporting this very perishable fruit have been gradually overcome and to-day the gigantic industry, supplying approximately 40,000,000 bunches to the United States annually, is on a secure basis and is still growing. The present war has somewhat checked the distribution of bananas to European countries, but importations in 1914 were sufficiently large to encourage the belief that consumption of bananas in those countries would soon rival that in the United States. In general, bananas are retailed by weight in this country, the present mainland market price being ten cents per pound. Sixty pounds of edible fruit would be a low estimate for the average bunch, so it is seen that the American consumers pay at least $200,000,000 annually for bananas.

The countries which furnish most of the bananas to the American and European markets are, in the order of their importance, Jamaica, Costa Rica, Colombia, Panama and the Canary Islands. Honduras, Guatemala, Nicaragua, and the Hawaiian Islands also export bananas in considerable quantities to the United States. Production of bananas in Cuba for export has declined in recent years owing to the extensive opening up of more suitable banana land in Central America.

The home consumption of bananas in some tropical American countries is of more importance than the export trade, since in many places it forms the principal sustenance of the natives. Porto Rico does not export bananas, but in the interior they are used largely as a substitute for bread. Practically every peon has a few dozen plants of bananas and plantains and in some places many acres are planted with some attempt at careful cultivation. The city of San Juan alone consumes about 3,000,000 dozens of bananas annually. In the country districts where the masses of people live, it takes the place of wheat, corn, and potatoes. This staple food is usually cooked in various ways by the hundreds of thousands of Porto Ricans for whom it is one of the main articles of diet.

2. FOOD VALUE

The banana is not merely an agreeable tasting fruit, but is also a food of high value. The composition of ripe fruit (3) is as follows: Water, 75.3 per cent; protein, 1.3 per cent; fat, 0.6 per cent; carbohydrates, 22.0 per cent; ash, 0.8 per cent; fuel value, 460 calories.

It is seen that the protein and fat are not high enough in proportion to the carbohydrates to make a perfectly balanced ration, but if supplemented with a small amount of beans, milk or meat, a diet of ripe bananas would suffice. The ash is composed of the phosphates, sulphates and chlorides of potash, soda, magnesia and lime, all of which serve useful purposes. The following table (25) shows the composition of the ash:

Composition of the ash of bananas

	per cent
Silica	2.19
Lime	1.82
Iron oxide	0.18
Phosphoric acid	7.68
Magnesia	6.45
Soda	15.11
Potash	43.55
Sulphur trioxide	3.26
Chlorine	7.23

Ripe bananas are easily digested, cheap, and wholesome. Owing to their many superior qualities, they have won a well deserved popularity in American and European markets. Any factor which threatens to curtail the production of this staple crop merits careful investigation, with a view towards eliminating the cause.

3. METHODS OF CULTIVATION

A few words in regard to the different methods of banana cultivation in various countries will serve to make clear the necessity for adopting procedures in the investigation of banana diseases, which vary somewhat from the usual practice among plant pathologists. The banana is a great perennial herb, requiring from twelve to twenty months from time of planting to attain maturity. The cultivated bananas, *Musa sapientum* and *Musa cavendishii*, have small, degenerate seeds, which are functionless and useless for propagating. New plants are started by means of suckers that arise from the tuberous underground stem or rhizome. These suckers are cut away from the mother plant when they are several months old. The top and roots are pruned off and the young "bulb" or "bit" is planted in an excavation made to receive it. The bulbs may be set out immediately or they may be allowed to dry for several days. In Hawaii, some planters place them in heaps and cover them lightly with trash, allowing them to remain so for a month before planting. The severing of the sucker from the mother plant, usually done with a sharp spade, results in a large wound, from six to ten inches in diameter, depending on the size of the rhizome. In Cuba many fields may be seen in which the tops of the suckers are not pruned off close, but rather at a height of 4 to 6 feet above the surface of the ground. Such bulbs are supposed to produce a mature plant in less time, but the root system is apt to be weakly developed and the plants may be blown down in moderately high winds. In Cuba, Porto Rico, Jamaica and the Hawaiian Islands, considerable preparation of the land is made. It is cleared, plowed, and in general, the best agricultural practice followed. The plants are placed uniformly in rows, the distance varying from 6 to 10 or more feet apart, both ways, depending on the variety. In Central America, however, when new banana land is opened up, the timber is cut and allowed to rot where it falls. Bulbs are merely thrown into holes dug among the fallen logs and nature is allowed to do the rest. The weeds and brush are cut with machetes two or three times a year, but further than that no care whatsoever is given the plants.

A banana plant produces but one bunch of fruit, which is harvested by cutting down the whole "stem," a sucker from the rootstock being allowed

to take its place. The sucker may have arisen a foot or more from the old plant, so it is evident that after some years the stool may have migrated to some distance from the original point where it was set out. This results in irregularity of the rows. In Central America, where no tillage is employed, this is a matter of indifference, but where the land is tilled with horse drawn implements, it is necessary to replant the fields to restore regularity. Lands must be suitably drained, and in arid regions, such as the south coast of Jamaica and the banana districts of Colombia, irrigation is employed.

4. VARIETAL SUSCEPTIBILITY

Banana wilt, the disease with which this paper is concerned, exhibits a decided variation in its ability to attack the different cultivated varieties of bananas. It is a curious fact, that in any particular region, this disease attacks most severely, and sometimes exclusively, the variety which is there most esteemed and therefore most widely planted. Other varieties may be planted in the immediate vicinity and optimum conditions for infection appear to exist, but they are not attacked. It is also interesting to note that varieties which are strongly attacked in one country appear to be resistant in another, although the disease is present in great abundance on some other variety. It will of course suggest itself immediately that we have here strains of the pathogene which exhibit biologic specialization.

Owing to the great number of varieties of the banana (estimated by some at more than a thousand) and the fact that in different countries the same varieties show variations, especially in regard to size and habit of growth, the problem is manifestly complicated. In addition, the botanical nomenclature is confused, and different local names are used for the varieties, even in different parts of the same country.

Typical cases of banana wilt have been observed by the writer affecting only four varieties, namely the Chamaluco in Porto Rico, the Manzana in Cuba, the Gros Michel in Cuba, Panama, Costa Rica, Guatemala,[2] Honduras,[2] and Jamaica, and the Red banana in Panama. The organism was easily isolated from all of these. In addition to the four varieties of *Musa sapientum* listed above as having proved susceptible, it has been suggested by one or two investigators and a number of planters, that other varieties and even other species of Musa are attacked. Among them are included the Dwarf or Chinese banana, *Musa cavendishii*, and the cultivated plantain, *Musa paradisiaca*. Dr. J. R. Johnston, Plant Patholo-

[2] Mr. L. W. Waters of the United Fruit Company gave the writer cultures of the pathogene from plants in Guatemala and Honduras.

gist of the Cuban Experiment Station at Santiago de las Vegas, reports having seen affected dwarf bananas in Panama in 1912, and to have isolated a species of Fusarium from affected tissues. I was unable to verify this in 1917, although scores of suspected plants were examined in the same region. However, there is no reason for doubting that this species may be affected under suitable conditions. The plantain is more vigorous than the banana, but occasionally plants are seen which are unthrifty for some reason or another, and planters are apt to ascribe the condition to "Panama Disease" or banana wilt. A number of such plants have been carefully examined, and in one of them a Fusarium was found occupying the tracheae in the pseudostem. Upon cultivation, however, it proved to be a member of the section Discolor of the genus Fusarium. These have also frequently been found associated with the true pathogene in the last stages of the disease and are to be regarded as secondary infections. The presence of Fusaria in the vascular tissue must therefore not be taken to mean that they are the causal agents.

II. THE DISEASE

1. NAMES

Various names have been applied to this disease, since it was first definitely recognized as such, among them being "the disease," "banana disease," "Panama disease," "banana blight," "banana wilt," "droop," "tired bananas," etc. in English-speaking countries, and "la enfermadad del platano," "la enfermadad" and "enfermadad Panama" in Spanish speaking countries. The name "Panama disease" first gained wide usage. It was probably originally used in Surinam on account of the resemblance of the banana disease which broke out there in 1906, to the disease which had played havoc in the plantations of Panama for some years prior to that time. It is improbable that the name "Panama disease" would be used first in Panama itself, since it would not there be particularly descriptive. The disease is by no means confined to Panama, but is widespread in the American tropics, being present in Central America, the West Indies, South America, the southern extremity of North America, also in the Hawaiian Islands and probably in the Old World.

A more satisfactory and descriptive name for the disease is "banana wilt," used in 1915 to describe the disease in Jamaica (Jamaica Department of Agriculture publication "The Law and the Orders Issued in Accordance Therewith with Regard to Diseases of Plants" p. 12, 1915.) Another less serious disease of bananas had previously been called "banana wilt" in Jamaica, but it was subsequently proved to be a bulb rot and not a true wilt. Furthermore, it is now widely known as the "Bonnygate disease,"

so there is very little risk of confusion if the name "banana wilt" is applied to our disease. It is very similar both in symptoms and in identity of the causal organism to the well-known "cotton wilt," "okra wilt," "tomato wilt," "cowpea wilt," "watermelon wilt," "potato wilt," etc., so it is believed that planters will accept the name which is in accordance with the best usage among plant pathologists.

2. HISTORY AND PREVIOUS LITERATURE

The first mention of this disease in the literature seems to have been made by Higgins (17) at Honolulu. It is a very meagre account, but identification of the associated fungus was made by a good mycologist, and since the disease has subsequently been proved to exist there (8, 31), it is probable that what Higgins observed was the true banana wilt.

It was in Panama and Costa Rica, however, that the disease first attracted wide attention on account of its destructive nature. McKenney (23) reports that the disease had attained alarming proportions in Panama and Costa Rica in 1904. He states, probably on the authority of planters, "As early as 1890 a few isolated spots were known to be affected, and from these the spread of the disease can be traced." The same statement was made to the writer in 1917 by a planter of long experience in Panama. McKenney was not able to isolate the causal organism, which is certainly surprising in view of the ease with which it may be cultivated, but his careful description of the symptoms leaves no doubt as to the identity of the disease.

Dr. Erwin F. Smith (28) at the same time described a disease of bananas from Cuba. He isolated a species of Fusarium from the discolored vessels of diseased material. Pure cultures of this fungus were inoculated into the midrib, leaf stalk and pseudo-stem of healthy banana plants at Washington. It was found that the organism would invade the vascular tissue of the inoculated plants to a distance of from 5 to 8 feet from the point of inoculation. The experiment was broken off before any secondary signs of the disease appeared and was not resumed. Doctor Smith named the organism which he isolated *Fusarium cubense*, but did not publish any technical description of it. He gives no description of the symptoms of the disease, since he had not at the time seen affected plants in Cuba. He states, however, that the disease is similar to a disease of bananas described by Earle in Jamaica in 1903 (11). This is certainly due to his not having seen banana blight in the field, since Earle's description shows conclusively that his disease has no connection with true banana wilt.

In 1911, Essed (13) describes what was undoubtedly banana wilt in Surinam, but his investigation bears all the earmarks of hurried work.

In 1912 Drost (10) also working in Surinam published a quite extensive account of his researches on what was unquestionably banana wilt. His conclusion that it is caused by an ascomycete, *Leptospora musae*, is based on insufficient evidence.

Research of a careful nature is indicated by the papers of Ashby (1, 2). He states that the banana wilt was first noticed in Jamaica in 1911. A good description of the symptoms are given. He obtained cultures of a Fusarium from banana tissues in a not far advanced stage of the disease, by the poured plate method. A very good description of the growth and appearance of this organism in pure cultures, the first to appear in print, is found in this paper. No inoculation experiments were attempted and no further contribution to our knowledge of the life history of the parasite is made. Recommendations for control of the disease, chiefly prophylactic, and rather drastic are set forth. It is suggested that the destruction of all plants within a radius of 66 feet of a case of banana wilt be accomplished and the area fenced and quarantined for an indefinite period.

Johnston (18) reports that banana wilt had become very destructive in the western end of Cuba in 1915. The Manzana variety was most subject to the disease and the Johnson (Gros Michel) was also strongly attacked.

Fawcett (14) gives the first published report of banana wilt in Porto Rico in 1910. In his brief account, he states merely that the disease is occasionally troublesome, and that two years previously a species of Fusarium was isolated from diseased materials. In a later paper (15) he records inoculation and control experiments, both of which gave negative results.

The present writer (5 and 6) records having isolated the organism from cases of banana wilt and also from the soil in banana plantations and adjacent fields in 1915. A detailed account of proof of causation, which will be repeated in the present paper, is also given.

Basu (4) notes the presence of a banana wilt in India, the symptoms of which agree very closely with the disease in the New World. The associated fungus is a vascular parasite of the genus Fusarium. It is described as having exterminated the most profitable variety of bananas at Chinsurah, while other varieties are not at all attacked.

Tryon (29) states that the disease has long been prevalent in Australia, but judging from his account it does not cause serious damage. His account is very incomplete and it is by no means certain that the disease he mentions is identical with ours. He speaks of a vascular parasite, causing discoloration of the bundles, and concludes from a consideration of its symptoms that it is identical with the "Panama disease."

Rijks (26) has recently described a banana disease in the Saleyer Islands, Dutch East Indies, which resembles banana wilt in many ways. The symptoms as described are not exactly typical of the disease in Central America and the West Indies but the differences may be due to the fact that the varieties of bananas grown in the two regions are not the same. No organism is mentioned in Rijks' paper.

Butler (7) and Shaw (32) report diseases of the banana in India in which a Fusarium is involved, but they are unmistakably rots and not true wilts. Many other papers on banana wilt and similar maladies have appeared but for the most part they are compilations, abstracts or reviews.

3. GEOGRAPHICAL OCCURRENCE

From the foregoing and other papers and from personal observations,[3] it appears that the disease has a very general distribution throughout the tropical regions of the world. With a few notable exceptions, probably due to climatic conditions, its range may be said to coincide with that of the cultivated banana. In the West Indies, it has been reported from Jamaica, Cuba, Porto Rico, Trinidad, and Barbados (9). The writer has made personal observations and comparisons of diseased plants and of the associated organism in the first three of these countries, and there is no reasonable uncertainty as to the identity of the disease. No doubt, this disease is present in other islands of the West Indies but on account of the limited extent of the banana industry and lack of agricultural investigations, it has not yet been reported from them.

In Central America, the disease is present in Costa Rica, Nicaragua, Guatemala, Honduras, and British Honduras. The writer found the disease in a most virulent form in Costa Rica (1917) and received cultures of the organism from Guatemala and Honduras from Mr. Waters of the United Fruit Company.

In North America, banana wilt has been reported from Mexico (24).

In South America, the disease has long been known. It first attracted wide attention in Panama early in the present century. No evidence that the disease has ever existed in Colombia, one of the greatest banana producing countries, can be found. In May and June, 1917, the writer made a diligent search for cases of the disease there but without success. This matter will be discussed later. The destructive nature and widespread occurrence of the disease in Surinam has already been noted.

[3] At the invitation of Dr. Erwin F. Smith, the writer made a trip to Cuba, Panama, Costa Rica, Colombia, and Jamaica in 1917 for the purpose of investigating this disease, on funds furnished by the Laboratory of Plant Pathology.

Evidence that it exists in India is fairly conclusive, and probably it is present in Australia and the Dutch East Indies as well.

On account of the great antiquity of the cultivated banana, and the scant records or lack of records concerned with its diseases, it would be difficult to trace the progress of the disease. That it was introduced into the Hawaiian Islands from Costa Rica seems certain, since the outbreak of disease there quickly followed the first importation of Gros Michel bananas from the latter into the former. The promiscuous shipping of stock from a common source into the various countries of Central and South America and the West Indies by large commercial interests having holdings in all of those countries, has been responsible for its wide distribution in tropical America. The intensive cultivation of bananas on the same land year in and year out has resulted in the disease becoming steadily worse. Where bananas are grown in a desultory way for home consumption the disease may be present but is never serious.

4. ECONOMIC IMPORTANCE

a. Extent of the losses

As early as 1910, banana wilt was regarded as one of the most important, if not the most important, fungus disease of cultivated plants in the American tropics. The extent of the industry which it threatened, its wide distribution, and apparently infectious nature alarmed banana planters almost to the same extent as did the dreaded Phylloxera the vineyardists of France some years ago. Although endemic, the outbreak in Panama in 1904 assumed the proportions of an epiphytotic. McKenney states (23) in 1910 that in Panama at least 15,000 to 20,000 acres of banana plantations had been abandoned and many thousands more were seriously affected, while in Costa Rica the damage had been even greater. Since that time the disease has continued to spread rapidly. In 1917 the writer visited the banana districts of Panama and found the older sections to be full of abandoned farms. In the Changuinola division, there is one tract of about 15,000 acres where the Gros Michel banana cannot be grown at all, which according to an official of the company had been their finest plantation five years previously. Another area of the same size was found to be practically abandoned, and the newer Sixaola division of 45,000 acres was already badly diseased. It has become the policy of the company to replant the abandoned areas with cacao and coconuts. Sometimes the Red banana, which is a little more resistant than the Gros Michel, is planted, and several harvests made before it in turn falls prey to the ravages of the parasite. In Costa Rica in 1917 conditions were little better. The newly opened farms show only an occasional dis-

eased plant, but it is only a matter of time when they too will become badly diseased. The seriousness of the situation is fully appreciated by the banana planters, and efforts have been made to ascertain the cause of the trouble and if possible to find a remedy. The United Fruit Company has established a small experiment station at Zent, Costa Rica where research is carried on in connection with this disease. It would be difficult to estimate the money loss in these two countries during the past decade, but it certainly amounts to many millions of dollars.

The history of the banana industry in the Dutch colony of Surinam is well-known to the student of tropical agriculture. In 1906, by agreement with the government the United Fruit Company supplied bulbs of the Gros Michel variety to the estates, and bound themselves to carry the product to the markets. Some cases of banana wilt were observed the next year, 1907, and by 1908 it had already done great damage. Still, in 1909, 648,636 bunches were exported from the colony, showing that the planters were determined to establish the industry. By 1910 every field was affected, and the attempt to grow the Gros Michel variety was abandoned entirely. Another variety, the Congo, was substituted but it did not meet the requirements of the market and it too was abandoned. A large majority of the planters, who had received advances from the government on the prospect of their crops, were ruined.

Last year, in the western end of Cuba, there was scarcely a plantation of Manzana bananas which did not show some signs of the disease, and many fields were seen in which every plant was affected. This variety is the most popular in Havana markets, but is not exported.

In recent years, the Chamaluco variety in Porto Rico has suffered severely from banana wilt. The plantation on the Experiment Station Grounds at Mayaguez is a veritable hotbed of infection. The suckers which come up from old stools appear healthy at first but they rarely bear a bunch of fruit. This variety, which is always cooked, has been in the past one of the main articles in the diet of the average Porto Rican.

Jamaica has not suffered any great direct loss on account of this disease, due in part to the vigilance of the government, which has established a quarantine of all affected areas, but also undoubtedly to its climate and soil which are quite different from those in Central America where the disease is rampant.

b. Nature of the losses

The nature of the losses due to banana wilt may be divided into three classes (1) injury to the fruit, (2) destruction of immature plants, and (3) depreciation of the value of the land. The first of these is the least serious

since affected plants do not usually bear a bunch. If, however, a bunch is "shot," it is apt to be small, the individual fruits are "bottle-necked," the flesh has a yellowish tinge and is pithy. Such a bunch would ripen unevenly and too rapidly and is always rejected by the inspectors on that account. It is a total loss. Destruction of the immature plants is the most serious phase of the disease. The plants wilt and die in enormous numbers, before any fruit is produced. The plant falls prostrate in a short time, but the rootstock is not immediately killed, and may send up fresh suckers. These will invariably become diseased also, and only rarely produce a marketable bunch.

The third type of loss, viz. depreciation in value of the land, is due to the fact that the causal organism may remain for long periods in the soil, and finally increase to such an extent that bananas can no longer be grown. Since in banana growing countries, the greatest profit is derived from these plants, and sometimes there is absolutely no sale for other products, it is evident that the land is greatly reduced in value or made utterly worthless.

5. SYMPTOMS

a. External signs of the disease

In a field where only occasional and isolated cases of the disease are found, indicating that the pathogene has not yet become thoroughly and uniformly distributed in the soil, the affected plants are not apt to exhibit any external signs of the disease until they are approaching maturity. Sometimes the disease is not made manifest until after the bunch has started to form. It is not uncommon to see a banana tree with a half formed bunch of fruit (plate XXIV, fig. 1), whose development has been arrested at that stage by the death of the plant. In these large plants, the symptoms are not unlike those brought about by severe drought. A typically diseased plant first shows a yellowing of the lower or outer leaf blades and petioles (plate XXI). The transition from the normal dark green color of the leaf to a vivid yellow is usually sudden and startling, and proceeds from the margin inward. Such leaves stand out conspicuously in a planting of bananas, the contrast with the healthy leaves making it easy to detect them even at a considerable distance. Their appearance, to one familiar with the disease is unmistakable, and they are not apt to be confused with any other trouble, if, as is usually the case, they appear when drought symptoms are not to be expected. A field was seen in Jamaica in 1917, in which practically all of the plants exhibited this symptom, but on examination no further evidence of disease was found. It was raining every day at the time, so drought was out of

the question. Mr. Cousins, director of agriculture, of the Jamaica Department of Agriculture said that the particular field had the same appearance every year and suggested that it was owing to the character of the soil. Such a condition must be exceedingly rare, since it is the only time it has come under observation in three years study of the disease. The yellowing of the lower, or outer leaves then is a practically unmistakable symptom of the disease. There is no risk of confusing it with the normal sloughing off of the lower leaves. These lower or older leaves gradually die off in a healthy plant, due to abandonment of the roots which supply the lower leaves. This natural phenomenon is characterized by the regularity with which the leaves die in sequence from below upward, and by the fact that at no stage is there a brilliant yellow color of the leaf, but only a gradual transition from dark green to brown.

Cases of banana wilt have been seen in which a younger leaf, possibly second or third from the bottom, was yellow, while those below it remained green, but usually the oldest (lowest) leaf is the first to go, then, in order, the next oldest and so on up to the topmost (youngest) leaf, which invariably remains green the longest.

The first attacked leaves begin to wilt almost immediately. Within a day or two the fleshy leaf stalk buckles at a point three or four inches from the pseudostem, and the leaf hangs pendant from this point. Sometimes buckling of the leaf-stalk or the large midrib takes place at any point out to the middle of the leaf blade or beyond, but the first mentioned condition is typical.

The leaf now rapidly withers and becomes brown (plate XXI). The process is rapidly repeated in the other leaves until the topmost or innermost (youngest) leaf is reached. If the fruit bud has not yet emerged, this leaf may be only partially or not at all unfurled. It is in a state of rapid growth and seems to resist a trifle longer than the other leaves. At any rate, diseased plants are very numerous, in which the youngest leaf stands erect and turgid, like a green lightning rod, for some time after the other leaves have succumbed. Finally, this leaf droops and withers, and the plant stands for a few days or weeks with the dry, brown leaves dangling and rattling in the wind (plate XXIV, fig. 2). A puff of wind, stronger than the rest, eventually sends the stately plant crashing to the earth, where it lies prostrate and quickly rots, due to secondary invasion by putrefactive organisms.

Another symptom of the malady, which is especially prominent in very heavily infected young plants, such as may be seen in abandoned fields where the disease is of long standing, is a decided dwarfing, or stunting of the entire plant. This dwarfing may range from a slight retardation in development, to the extreme condition, observed in the western end of

Cuba, where the plants do not grow to be more than one or two feet tall. The pseudostems of these diminutive plants have a constricted, or "hide-bound" appearance and the margin of the leaves may be undulating or the whole leaf curved or distorted in various ways. The leaves do not wilt so rapidly as those of larger plants, nor is the yellow color so conspicuous. Exactly the same signs were seen in plants growing in soil artificially inoculated heavily with the causal organism at Mayagüez, P. R.

As stated above, this dwarfing may be less decided, and this is more often the case. The plants may attain a third or a half of their normal size in the time required for complete development. This condition means that the plants arose from diseased bulbs or were strongly infected immediately after planting. The leaves become yellow and wilt just as do the leaves of larger plants.

Another symptom that frequently accompanies stunting, is a longitudinal splitting of the outer leaf bases (plate XXV, figs. 1 and 2, and plate XXVI, fig. 1) which form the pseudo-stems. The split may extend all the way from the rhizome, at the base or surface of the ground, to the collar or place where the leaves diverge, or it may be more limited in extent. Only the outer leaf bases may be involved, or the opening may extend to the center of the pseudo-stem, in which case it may happen that the young leaf becomes diverted from its course up through the center, and projects out through the split. This condition also has been produced in artificially inoculated plants (plate XXVI, fig. 2).

All of the foregoing symptoms have been observed in the varieties Chamaluco, Manzana, Gros Michel and Red, in Porto Rico, Cuba, Costa Rica and Panama respectively and in the Gros Michel in Jamaica. From the literature (10) it is learned that they occur in Surinam also.

If a bunch of fruit has formed on an attacked plant, it also shows signs of the disease. Since, in the main, the differences between the various varieties are most marked in the characters of the fruit, the bunches of the several susceptible varieties exhibit symptoms that vary somewhat. In general, however, it may be said that the bunch will be found to be small. Development may be completely arrested after a few hands have been formed, or if a normal number of hands are produced, the individual fingers are small and "bottle-necked" i.e. the ovary is constricted or "pinched-in" at the calyx end. The fingers do not ripen uniformly. Occasional fingers, scattered throughout the bunch become yellow rapidly. The flesh is inclined to be pithy, acrid and yellowish and judging from the taste, the starch is not converted into the soluble sugars. Since a bunch is rarely produced, this symptom is of little value in diagnosis.

b. Internal signs of the disease

Healthy banana tissue, both in the rhizome and pseudostem is almost dead white when first cut open (plate XXII, figs. 1 and 2). After a few minutes, especially if it has been cut with a steel knife, a purplish discoloration will appear uniformly distributed over the cut surface, due to the presence of oxidizing enzymes. If a plant in the incipient stages of banana wilt be removed from the ground and a transverse cut made in the lower part of the rhizome, it presents a quite different appearance. (plate XXIII, fig. 2.) Within the stele, small dots and irregular threads of a yellowish or very light brown color are seen, either distributed evenly over the whole cut surface, or more frequently arranged in a band just inside of the endodermis. Sometimes they are localized in one or more patches just within the endodermis. Such a patch at a more advanced stage is shown in plate XXIX, figure 2. Upon examination these dots and lines are made out to be the discolored vascular bundles, which in this situation run in every conceivable direction, so that there may be transverse, longitudinal, and oblique sections of bundles in the same plane. Upon cutting successive sections of the rhizome towards the apex, it is found that the discoloration gradually becomes less pronounced in an upward direction and finally disappears altogether. If a plant in a somewhat later stage of the disease is selected and the operation repeated, it will be found that the bundles in the lower part of the rhizome are more deeply stained, and more numerous, the color ranging from reddish to reddish brown. A few scattered discolored bundles may be seen in the cortex. Successive sections cut at intervals towards the apex reveals discolored bundles in the stele extending upward towards the base of the pseudo-stem. At various points near the apex of the stele, some of these discolored bundles are seen to pass through the endodermis, and traverse the cortex in an upward and outward direction, passing thence directly upward into the leaf-bases. During their passage through the cortex where they form the leaf-traces, these discolored bundles are very conspicuous in the otherwise healthy and white cortical tissue. The previously mentioned scanty diseased bundles in the cortex represent these leaf traces. The most successful method of following their course is to cut a diseased plant in half longitudinally (plate XXVII, fig. 1). Some of them will be revealed, and by carefully excavating the surrounding tissue with a sharp scalpel, one of them can be followed continuously from deep in the stele, across the cortex and far up into the leaf petioles (plate XXVII, fig. 2). Here the color gradually becomes lighter as they progress towards the collar and eventually, in this stage of the disease, the discoloration is no longer found.

In such a plant, if the pseudo-stem is cut off transversely near the base, only cross sections of discolored vascular bundles will be found (plate XXIII, fig. 1). The bundles here are straight and vertical, and are arranged in concentric arcs, conforming to the clasping leaf bases of which the pseudo-stem is composed.

At a quite advanced stage of the disease, say when most of the leaves are wilted, a cross section of the rhizome shows the vascular bundles of the stele to have changed in color, from reddish brown to purple or even black and to be so numerous that most of the stelar tissue, especially that near the periphery, is quite dark in color (plate XXVIII, fig. 1). The cortex is still white and healthy looking excepting for the few scattered discolored bundles forming the leaf-traces. Somewhat later the entire stele becomes entirely black (plate XXVIII, fig. 2). Secondary rots have set in by this time and it is more difficult to isolate the causal organism from such tissue. As a result of such rots, which are occasionally putrefactive, a disagreeable odor is sometimes given off. It has been noted also that a yellowish or brownish discoloration of part or all of the stelar tissues takes place when putrefaction sets in but this is of infrequent occurrence. A cross section of the pseudo-stem now shows reddish, purplish or black vascular bundles at any point up to the crown, and even in the leaf-stalk or midrib.

At this point it may be mentioned that certain more or less uniform differences exist in the appearance of the diseased pseudo-stem cross sections in the several attacked varieties. In all of them, the leaf bases at the center of the pseudo-stem remain healthy after the bundles of the outer ones have become discolored. This is to be expected when we recall that under "gross appearance, etc." it was mentioned that the progress of the disease is from the lower (outer) leaves to the upper (inner) ones. In the Gros Michel, this phenomenon is not so conspicuous, that is, there is a more even distribution of the discolored bundles throughout the cut surface of the pseudo-stem (text fig. 1).

With the Manzana variety a more pronounced delay of invasion of the inner leaves is seen. The condition in the Chamaluco is still more complicated, for in typical cases the cross section shows a decided band of diseased tissue, with healthy tissue both within and without (plate XXIX, fig. 1). Occasional discolored bundles may be found, it is true, on both sides of the band, but the tissues there are decidedly less attacked.

Passing to the roots, which of course originate at the stele, and pass thence through the thick, fleshy cortex and so into the soil, symptoms of the disease are exhibited in various ways. The main roots are fleshy and of uniform diameter throughout. From these small thread-like roots arise laterally, and branch profusely. On the thread-like roots and back

of the growing tip of the main roots are borne the root hairs. The primary meristem occupies only a very small portion behind the root cap, and is very tender. The main roots do not seem to adapt themselves well to irregularities in the soil, but upon meeting a stone or other obstruction, the tips by rapid cell division and growth push into it and are crushed. The root dies back and a lateral thread-like root behind the apex takes the lead, and becomes the main root. It is not surprising that many roots are seen which are dead and decayed. Unless complications have set in, this must not be taken to indicate a diseased condition.

FIG. 1. TRANSVERSE SECTION OF PSEUDOSTEM OF GROS MICHEL VARIETY IN JAMAICA

Notice the rather even distribution of discolored vascular bundles

Nematodes, insects and other agencies also may injure the roots. However, unmistakable evidence of disease in the roots may be found in connection with wilt. Blackened roots, close to the bulb and extending into diseased portions of the stele are frequently found (plate XXVII, fig. 1) and this condition has been proved to be due to the banana wilt organism (see discussion under "life history").

It may be proper to discuss here some variations in the situation of primary diseased stelar tissue. An explanation for the condition will be reserved for discussion later. In dissecting hundreds of diseased plants, it is seen that there are two regions where the disease apparently first gains

a foothold: (1) at the cut surface of the rhizome, where the sucker was cut away from the mother plant resulting in general infection (plate XXVIII, fig. 1) and (2) at the side of the bulb, where no wound is present (plate XXIX, fig. 2). The evidence for this is deduced from the fact that the diseased tissue in the early stages of the malady is confined to either or both of those regions, and in more advanced stages the tissue in those regions is manifestly more strongly attacked than elsewhere, as can be readily seen by a glance at the illustration. Where the diseased stelar tissue originates at the side of the bulb, a diseased root can invariably be found leading out from it into the soil.

6. ETIOLOGY

a. Morphology of the causal organism

The causal organism is *Fusarium cubense* E. F. Smith amended E. W. Brandes.

1. Sporodochia and conidia. Sporodochia are found at the surface of leaf stalks, and blades, sometimes also of leaf bases, emerging through the stomatal openings (plate XXXIV, fig. 2). They are most numerous on the upper epidermis of leaf stalks at the point where they diverge from the pseudo-stem. On account of their location in stomata, they naturally stand separate, and are not joined by any type of stroma. The sporodochium arises from a globose mass of pseudoparenchymatous tissue 26 to 30 μ in diameter which entirely occupies and distends the substomatal cavity. This pseudoparenchymatous tissue is composed of quite large, thin walled, isodiametric cells. The cells vary from 2.6 μ to 10.4 μ in diameter. This fungus tissue wedges the guard cells apart at the aperture to an average width of 21 μ. At this point the cells are no longer isodiametric, but are elongated in the direction of outward growth, and are united in filaments. Occasional large globose, bladder-like cells, up to 10 μ in diameter are found intercalated in the filaments or attached terminally, the tissue here being somewhat vesiculose. The individual conidiophores arise just where the fruiting body emerges from the stomata, and diverge from one another so that the somewhat loose aerial tissue flares out in all directions and is roughly obovate. Conidiophores are verticillately branched, with two and occasionally three one-celled branches in a whorl. The average length of conidiophores is 70 μ, and of the lateral branches about 14 μ. The largest diameter of both main stalk and branches is 4 μ. The apical ends of the lateral branches taper abruptly, but the tip of the main axis is drawn out into a slender needle-like end. The main axis is about seven times septate, and the whorls of branches arise at the extreme upper end of cells of the main axis.

Usually three whorls of lateral branches are found on a conidiophore. Conidia are borne at the apical ends of both the lateral branches and the main stalk. These are of two distinct types. At first 0- and 1-septate microconidia are born in great abundance. They are ovate, or somewhat elongated, and range from 5 to 7 μ by 2.5 to 3 μ. A few abortive, 1- and 2-septate sickle-shaped conidia are found interspersed among the microconidia. These vary greatly in size, approaching the microconidia on the one hand and normal macroconidia on the other. Finally, the large, hyaline pedicillate, sickle-shaped macroconidia are produced (plate XXXI, fig. 1). More than 95 per cent of them are 3-septate, a few 4- and 5-septate individuals being found. They vary from 22 to 36 μ in length, and from 4 to 5 μ in their largest diameter. The mature sporodochia are dry and powdery. They appear white by reflected light. On account of their minute size and the rather restricted area upon which they occur normally on the host, it is not surprising that they have never before been reported. Pure cultures obtained from these sporodochia by the loop dilution method do not differ from those obtained by plating out diseased internal tissue.

2. Development of sporodochia. Tangential, radial and transverse serial sections of affected leaf stalks, stained with safranin and haematoxylin, show sporodochia in all stages of development. They arise from intracellular mycelium in the epidermal and subepidermal cells of the host (plate XXXIV, fig. 1). In the vicinity of the substomatal cavity the mycelium possesses occasional bladder-like cells, which are terminal or intercalated. Such cells are not seen in the vegetative forms of the fungus in banana tissue. This mycelium penetrates the substomatal cavity, where it loses its filamentous character altogether. The first cells to enter the cavity are large and bulbous. They divide rapidly, producing cells of a like nature, until the whole cavity is distended with pseudoparenchyma. The growth is very irregular, and there is no suggestion of chains of cells. At first this mass of fungus tissue conforms to the original shape of the cavity, but by the pressure of its growth, exerted in all directions, it soon becomes globose. Before this portion of the sporodochium has attained its maximum size, strands of hyphæ push out through the stomatal aperture. Successive outgrowths result in a wedging apart of the guard cells in a short interval of time. These same strands of hyphæ continue to grow apically, by cutting off end cells. Just subsequent to passing through the aperture, they diverge, and become the conidiophores.

3. Mycelium. The mycelium is intracellular. Evidence of intercellular mycelium has been seen in a few sections, especially near the point of penetration in roots and stele, but this type seems to be rare. In the cor-

tical cells of the roots, and also in the xylem, where they occupy the lumena of vessels, the strands are hyaline and septate, the septa being spaced at intervals of about 14 μ on the average. There are here no constrictions at the septa, the tubes being about 2.6 μ wide and of uniform diameter throughout. The contents are densely granular. In these regions the mycelium takes an irregular course, but in the vessels of the leaf bases it is more commonly straight. In the parenchymatous cells of the leaf-stalk and blade, just previous to sporodochium formation, the fungus cells are swollen and barrel-shaped. The hyphæ are more richly septate, with decided constrictions at the septa. Occasionally an enormously distended cell will be found, perhaps 8–9 μ in diameter. The mycelium is in general a trifle larger here than elsewhere, averaging 3 to 4 μ in diameter. The large bladder-like cells occur terminally or less frequently intercalated between other cells of the hyphal strand. Oil drops and other inclusions are more noticeable in mycelium of these regions.

4. Cultural characteristics of the causal organism. It is not proposed to go deeply into the vexed question of separating the species and varieties of Fusarium on their morphological and physiological characters in pure culture. The limitations of such attempts have already become evident. We do not believe that it is possible at present to separate *Fusarium cubense* from nearly related forms on this basis. Possibly methods may be devised in the future by which it can be accomplished. The following data will serve mainly to show the near relationship between *Fusarium cubense* and other species, notably *F. vasinfectum.* This organism has been under continuous observation in pure culture for the past three years. The study has served to show that the same strain, originally from a single-spore isolation will vary considerably with the age of the culture or the kind of inoculum used (mycelium, conidia, sclerotia). Even two sub-cultures will vary somewhat under apparently identical conditions. The following descriptions are of freshly isolated cultures, material that seems to vary the least.

The organism grows rapidly and luxuriantly on potato plugs, producing dead white, aerial mycelium in great abundance. Microconidia are formed in enormous numbers in about two days, being abstricted at the tips of the short lateral branches of conidiophores. They are one-celled, and variable in size, ranging from 5 to 7 μ by 2.5 to 3 μ in size. Many of them accumulate at the fertile tips of hyphæ, being held in globose heads by a gelatinous or watery matrix. Occasional normal macroconidia may be found in such a culture, but they are very rare. Transition forms between the microconidia and macroconidia are numerous. They are 0- 1- and 2-septate and may be straight or curved. In five or six days, small masses of hard, flesh-colored, plectenchymatic tissue develop at the surface of

the substratum. These are smaller than a pin head when first seen. They develop rather slowly and become indigo blue in color when ten to twelve days old. These sclerotia range from one to four mm. in diameter and are irregular and nodule-like.

On potato agar slants, a more spreading growth takes place. The mycelium penetrates deeply into the medium and less aerial growth takes place, although it is considerable sometimes. Cultures may make a low slimy growth with only a little aerial mycelium. Besides the types of conidia found on potato plug cultures, so-called "sporodochia" are formed on various agar media if the air is moist. These are wart-like heaps or limited, more or less convex layers of macroconidia borne on closely packed conidiophores. They are salmon colored and range from 2 to 5 mm. in diameter. When the culture has become somewhat dry, chlamydospores are produced, arising from cells of the mycelium or macrospores. They occur singly, in pairs, or in chains, and may be terminal or intercalary. They become thick-walled when old. Individual chlamydospores are globose to short oval, and in size are 5.5 to 6 μ by 6 to 7 μ. Paired or catenulate chlamydospores are usually a little smaller.

On steamed rice grains, a luxuriant growth of white mycelium is made which penetrates to the bottom of the test tube. Very soon the substratum becomes colored a delicate Hermosa pink (Ridgway). The color gradually deepens, but usually not uniformly, but rather is streaked or blotched and varies from Hermosa pink to spectrum red. Vinaceous tinges occur later in all cultures and some of them become violet purple. These color changes have been observed in cultures from all varieties of bananas secured in Porto Rico, Cuba, Panama, Costa Rica, Guatemala, Honduras and Jamaica. When old and dry, the white mycelium of cultures obscures the color of the substratum. Small bits of white to flesh-colored plectenchyma are seen scattered through the culture and adhering firmly to the glass walls of the test tubes. These have not been observed to become indigo blue, as do similar bodies in potato slab cultures. Microconidia are formed on rice, but no macroconidia. A quite noticeable agreeable odor is generated by some strains of *F. cubense* when grown on this medium. When requests have been made of various people to identify the odor, it has been described as similar to the odor of "orange peel," "ripe watermelon," "cucumbers," "fermented grapes," "lilacs," etc.

On stems of *Melilotus alba*, the fungus first makes an abundant growth of white aerial mycelium. Within a few days, however, a nearly continuous slimy layer of macroconidia will form on a considerable area of the stem. This layer may have a smooth surface, or may be lumpy or nodule-like, the irregularities of topography being caused by the more abundant production of conidia at certain points. This type of fructification has been referred to as a "pionnotes." The spores are salmon colored in mass.

b. Physiology of the causal organism

1. Isolation. The organism is easily isolated in various ways. The chief requisite is the use of tissue in the incipient stages of disease. For rapid work, such tissue cut from the root, rhizome, leaf-base, or leaf stalk under aseptic conditions, and planted in sterile media will give a pure culture of the parasite. If the disease is far advanced, bacterial and even fungus contaminations are likely to occur. If only advanced cases are available, bacterial growth may be inhibited by adding to the media 2 cc. of a 10 per cent solution of lactic acid.[4]

For refined work it is necessary to obtain single-spore strains of the organism. Various methods of doing this successfully are available. Tissue such as would be used for direct plantings may be removed with the same precautions and crushed in sterile melted agar, after which it is poured into petri dishes. Many colonies will arise from microconidia present in the vessels. If clear media and thin-bottomed petri dishes are used, the conidia may be located with the microscope through the bottom of the plate and marked with India ink. A colony resulting from the germination and growth of a single conidium can be lifted from the plate with a sterile platinum spatula, and transferred to fresh sterile media.

The single-spore strain is thus obtained directly. Conidia from a colony resulting from direct planting may be stirred into sterile melted agar, after which point the above mentioned process is followed.

Conidia borne externally on sporodochia can be scraped off with a sterile scalpel, stirred into melted acid agar, and plates poured. Single conidia are easily located in the same manner and the resulting colonies transferred.

Isolation of the organism from the soil is accomplished by taking a small particle of soil from below the surface, at any depth from one to thirty inches, under aseptic conditions (30) and transferring it to acid agar or steamed rice tubes. By this method of direct planting, contaminations may occur, but in heavily infested soil, the pathogene usually outgrows the other organisms present. Single spore strains are obtained as above from these cultures. If it is desired to determine the number of conidia in the soil, a measured quantity of soil is taken and dilution plates poured. Media for this purpose should be acid to discourage bacteria, and starchy, so that the pathogene may be quickly recognized by its characteristic color production.

It is realized that the behavior of a fungus in pure culture under controlled conditions is at best only an indication of what may be expected

[4] This amount of acid in 10 cc. of potato agar will permit of a somewhat restricted growth of the Fusarium and will prevent the development of the common bacteria.

under natural conditions. Insofar as deductions to be drawn from the relation of the organism to moisture, heat, light, etc. are concerned, if made with the end in view of offering explanations for the various phenomena exhibited by the organism during its parasitic existence in the host or during saprogenesis in the soil, they are of limited applicability and should be accepted with reserve. However, knowledge of this kind is of some service in this connection, and to a certain extent may also be of some value in delimiting the organism concerned from some of its close allies. A few experiments have been conducted with *Fusarium cubense* to ascertain its response to varied conditions of heat, moisture, light and oxygen supply.

2. Relation to heat. The thermal death point of macroconidia obtained from fresh pionnotes on *Melilotus alba* stems was determined by the method in use by bacteriologists. A spore suspension was made in sterile distilled water, and drawn up into capillary glass tubes six inches long. These were sealed by holding the ends in the flame of a Bunsen burner for a few seconds. The tubes with included spores were then exposed for ten minutes to constant temperatures in the water bath, ranging from 40 to 55°C., the series varying by intervals of one degree. The water in the water bath was maintained at any desired temperature by the use of Novy's thermo-regulator, and was continuously agitated to obviate the inequality of temperature at various points due to convection currents. After the exposure, one end of the capillary tube was broken off, and the contents "shot" into a dish of sterile agar by applying a flame to the closed end. Germination of the conidia was observed through the bottom of the petri dish. The average result of three trials is as follows.

TEMPERATURE	PER CENT OF GERMINATION AFTER		
	12 hours	24 hours	48 hours
°C.			
42	99+	99+	99+
43	99+	99+	99+
44	99+	99+	99+
45	90	94	95
46	40	50	50
47	15	40	40
48	15	30	30
49	8	30	30
50	5	30	30
51	0	20	20
52	0	20	20
53	0	15	15
54	0	1	1
55	0		1
56	0	0	0

According to this arbitrary method, the thermal death point is 56°C., which is remarkably high. A decided decrease in percentage of germination at temperatures of 46°C. and above is to be observed. At temperatures above 50°C. germination is of a type that is decidedly abnormal. The germ-tube is thick and makes a slow, feeble growth.

Sustained exposure to heat causes death at much lower temperatures. Using the same technique it was found that twenty-four hours exposure to 42°C. killed all of the spores, and only a small percentage germinated after twenty-four hours at 38°C.

3. Relation to moisture. Freshly produced macroconidia and microconidia are very tender to desiccation. An experiment was conducted to determine how long such spores would withstand drying on clean cover glasses at ordinary atmospheric humidity. A suspension of spores in distilled water was made up, using material from pionnotes on *Melilotus alba* stems, and drops of the suspension were placed on clean sterile cover slips. The water evaporated quickly leaving a cloudy mass of air dry spores on the glass. The slips were placed away from the dust in a covered culture dish. At intervals of two hours, a slip was removed, and a drop of sterile distilled water placed on the spores. It was then inverted over a Van Tieghem ring and set away. Most of the conidia failed to germinate in distilled water after drying for a period of four to seven hours. There were still a few feeble germinations at twenty hours but none at twenty-two hours. The result of this experiment is outlined in the following table.

PERIOD OF DESICCATION	GERMINATION	PERIOD OF DESICCATION	GERMINATION
hours	*per cent*	*hours*	*per cent*
0	80	14	4
2	80	16	2
4	80	18	1
6	40	20	1
8	15	22	0
10	4	24	0
12	4		

Possibly a higher per cent of germination could be obtained by substituting some nutrient solution for distilled water. Spores from cultures that are apparently very dry and have been so for weeks will sometimes germinate readily.

Chlamydospores and sclerotia are quite resistant to desiccation. They will germinate after being air dry for five or six months, possibly even longer, but no evidence that they will resume growth after six months is

available. Since these bodies have not been found in nature, no exact experiments have been performed for the purpose of determining their longevity under dry conditions.

In old dry cultures on bean pods, chlamydospores are sometimes found to be absent, but the ordinary mycelium, which has become quite thick walled, has been known to resume growth after being quite dry for four months.

4. Relation to light. Attempts to vary the growth of the organism by controlling the amount of light available for it have had slight success. Light-tight cases were made of black opaque photographic paper in the following way. Two open paper cylinders were made by gluing the opposite edges of sheets together. Both were eleven inches long, but one was 4 inches and the other 6 inches in diameter, so that the smaller would fit inside of the larger. Caps for both ends were made by gluing the rim of a short cylinder, 5 inches in diameter to a round disc of the same material. The cup was so constructed that the attached short cylinder would fit between the two 11-inch cylinders. The rims of the long cylinders were toothed to insure circulation of air, and all seams made light-tight with a mixture of lamp-black and paraffine.

Similar cases were made out of transparent tracing cloth. Newly isolated cultures on potato plugs and steamed rice were placed in all of the cases, and the latter were placed in front of a south window in direct sunlight. All conditions of temperature, humidity and aeration were thus exactly the same, so if light exercised any limiting influence, it would be here determined.

After ten days the cultures were removed from the cases and examined. All had made an abundant vegetative growth. There was no perceptible difference in amount between the cultures in absolute darkness and those in strong diffused light. Macroconidia, microconidia and chlamydospores had been produced in equal numbers in all of the tubes. The sclerotia on potato plugs were identical. The only observed difference was a slight variation in color production in the rice cultures. Those in the light had slightly more prominent streaks of a vinaceous color in the otherwise pink background.

5. Relation to oxygen supply. To determine the part played by free oxygen in the development of the fungus, Erlenmeyer flasks of various sizes ranging in capacity from 10 to 1000 cc. were filled to a uniform depth with corn meal and sterilized in the autoclave. Each flask was then inoculated with spores from a newly isolated culture by a single stab at the center. When the cotton plugs were replaced they were immediately sealed securely with paraffin.

Readings were taken before the edges of colonies in the smallest flasks reached the glass walls of the vessels to avoid the criticism that any restriction of development was due to limited space for growth. The edges of the colonies in the smallest flasks were still 6 to 7 mm. from the walls when readings were made. Results:

Effect of oxygen on growth of organism; time nine days

FLASK CAPACITY	DIAMETER OF COLONY	CHARACTER OF GROWTH
cc.	*mm.*	
10	15	Flat effuse
10	15	Flat effuse
10	15	Flat effuse
100	41	Raised
100	38	Raised
100	40	Raised
250	55	Aerial
250	58	Aerial
250	59	Aerial
500	75	Aerial
500	65	Aerial
500	72	Aerial
1000	90	Fluffy aerial
1000	70	Fluffy aerial
1000	80	Fluffy aerial

Color production in the media was the same in all cases, a deep vinaceous red. An equal production of conidia took place in all cases. In the absence of any other logical explanation for the limited growth in the small flasks it seems probable that the more rapid growth in the large flasks was due to more abundant oxygen supply. Inhibition due to less chance of wide diffusion of autotoxic excretions seems less reasonable.

6. Excretions of the fungus. When a culture stands for some time under very humid conditions, large drops of liquid gather in the aerial mycelium. Sometimes this is so extensive that the drops run together and form a continuous film over the surface so that the culture resembles wet fur. This phenomenon has been termed transpiration, and it is believed to be similar in function to transpiration in the higher plants.

For the most part we have only indirect evidence that excretions are made by mycelium below the surface of the substratum. Several times it has been mentioned that starchy media such as rice, corn meal, etc., are discolored where they are in contact with the mycelium of *F. cubense*. In the host tissue also various colors are produced. The study of color production, a characteristic which the organism has in common with

various other fungi, is of general interest and constitutes a separate problem. Undoubtedly it is due to the excretion of substances by the fungus into the substratum. Attempts to vary color production by growing the organism in acid and alkaline media have failed.

It is known that some strains of *F. cubense* generate propionic aldehyde when grown in certain synthetic liquid media (19), and the aromatic odor which is detected when the organism is grown in these media is ascribed to this substance. The same odor occurs when the organism is grown on steamed rice. It has been suggested (Lathrop) that this substance may be responsible for the pathologic effect on the host, on account of the deleterious effect of aldehydes on plant growth. This seems improbable however, since there are strains of *F. cubense,* which are parasites of the same aggressive type, but no odor accompanies their growth on these same media, and probably no aldehydes are produced.

Experiments have been conducted by the writer, however, that seem to indicate that there is a relation between excretion of toxic substances by the parasite, and the symptoms exhibited by attacked plants. It has been noted (1) that the fungus tissue in the lumena of vessels is not present in quantity sufficient to cause serious obstruction to the passage of water. There are exceptions to this but as a rule it is true. Wilting, then, must be caused by some other means than the mechanical plugging of the lumena of vessels and the resultant restriction of water supply to the leaves. Cultures of *F. cubense* from Jamaica and Porto Rico were grown in 500 cc. of Richards solution[5] in 1 litre Erlenmeyer flask for two weeks. The fungus made a rapid growth and occupied most of the available space in the liquid at the end of that time. The cultures were then filtered under aseptic conditions and the filtrate was poured into 250 cc. flasks, about 200 cc. being put into each. An equal amount of the same solution that had merely been sterilized, but not inoculated was placed in each of another series of 250 cc. flasks. Another series was filled with sterile distilled water. The stems of seeding plants of buckwheat about ten inches tall were now cut off under water at the surface of the ground, and the tops quickly transferred to the flasks containing the various liquids. One plant was placed in each flask so that the cut ends were deeply immersed. The experiment was performed in a greenhouse at noon March 23, 1917, at Ithaca, New York. A bright sun was shining at the time. Within five minutes indications of wilting could be seen in the plants immersed in the filtrate from cultures. In fifteen minutes the plants were very noticeably wilted, and in one-half

[5] Composition of Richard's solution: 10 grams KNO_3, 5 grams KH_2PO_4, 2.5 grams $MgSO_4$, 20 mgm. $FeCl_3$, 50 grams cane sugar, 1000 cc. water.

hour the leaves hung perfectly limp, and even the tips of the stems drooped to one side. The check plants, both in uninoculated Richards solution and in water remained perfectly turgid for 24 hours after the experiment was started.

The experiment was repeated, using bean seedlings in place of buckwheat, with the same results (text fig. 2), except that the time required for wilting was somewhat longer, it being fully two hours before the plants were decidedly wilted.

Banana leaves were now used with the result that it required fully 24 hours for the wilting to become perceptible. The banana leaf blade and leaf stalk are composed of leathery and fleshy tissue respectively and it is hardly to be expected that the leaf would wilt as quickly as the rela-

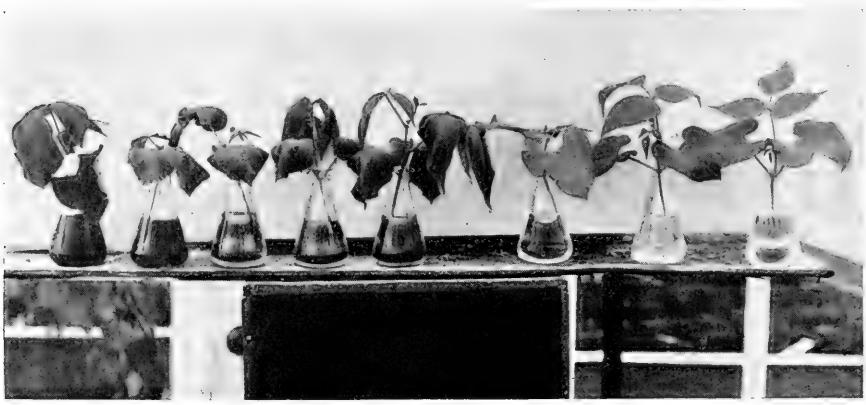

FIG. 2. WILTING OF BEAN PLANTS BY TOXIC EXCRETION OF FUSARIUM CUBENSE

The five plants on the left are immersed in the filtrate from a culture growing in Uschinsky's solution. The next two plants are in plain sterile Uschinsky's solution, and the one on the extreme right is in water.

tively soft, tender tissues of the other plants. The time required in this case was of course long enough to permit the growth of other organisms in the media. This is unfortunate, but sterilization of the banana leaf stalk cannot be accomplished without injuring it and otherwise interfering with the experiment. Results in the other two cases, however, convinces the writer that the wilting here was due to the same causes.

It will immediately occur to any student that the wilting may have been due to increased osmotic pressure in the filtrates from cultures. It is true that before the cane sugar, a dissaccharide, of the culture solution is available to the fungus, it must be inverted by some excreted enzyme such as invertase, and the resulting monosaccharides, glucose and fructose, would have approximately double the osmotic pressure of the original sugar so-

lution. It was then decided to use another synthetic culture medium in which the carbon is supplied in some other form and in small amounts. Uschinsky's solution, in which the organic matter consists of small amounts of ammonium lactate and sodium asparaginate was next used, but with precisely the same results. Goss (16) by employing slightly different methods, came to the conclusion that the wilt of potatoes caused by *Fusarium oxysporum*, is brought about by the action of toxic substances excreted by the fungus. In the case of the banana disease, wilting is not due to plugging of the vessels by mycelium, but is probably the result of toxic excretions by the fungus.

c. Pathogenicity

On account of its constant association with the disease, and on account of the evidence put forth as a result of some rather doubtful experiments, the ability of *F. cubense* to cause the disease has been assumed for some time. In previously recorded experiments by Drost (10), inoculated plants were grown under very unnatural conditions in which normal development could not take place. What is worse, it is evident that cultures used for inoculating the plants were not pure, since "pycnidia" and "perithecia" are described as arising from them. These cultures were obtained by direct plantings of diseased tissue and it is probable that they were contaminated.

In the fall of 1915, at a time when the writer was not aware that work had previously been done on this disease, experiments were started at Mayaguez, Porto Rico in an attempt to prove the causal relation of the associated fungus. As a result of those experiments, it was possible to furnish formal proof of the pathogenicity of the parasite, and the results were published (6) in the annual report of the Porto Rico Agricultural Experiment Station for 1916. The principal experiment was conducted as follows: Thirty cylindrical cement tiles, 3 feet in diameter and 4 feet deep were constructed and sunk into the ground in prepared excavations so that the rim projected 4 inches above the surface of the ground. Each tile had a "collar" about 6 inches wide 4 inches below the rim which rested on the surface to prevent settling. The tiles were all filled with a mixture of clay loam and river sand. The soil in twenty of the tiles was sterilized with live steam from a specially constructed apparatus. (Text fig. 3.) This consisted of a ramification of pipes with small perforations in three rows lengthwise of the pipe at intervals of $\frac{1}{2}$ inch in the rows. The pipes were so disposed that the soil in no part of the tile was more than 6 inches from a jet of steam issuing at 80 pounds pressure. The pipes were connected by means of steam hose to a boiler capable of gen-

erating and discharging large quantities of steam. The whole apparatus was mounted on a stone boat so that it could be moved to any desired point by a pair of oxen. In sterilizing the soil, the rigid series of pipes was driven into the rather light soil, and the steam turned on when it had attained a pressure of 80 pounds. This was maintained for two hours after which the steam was turned off and the pipes removed with sterile wrenches. Twenty minutes after the steam was turned on the soil at

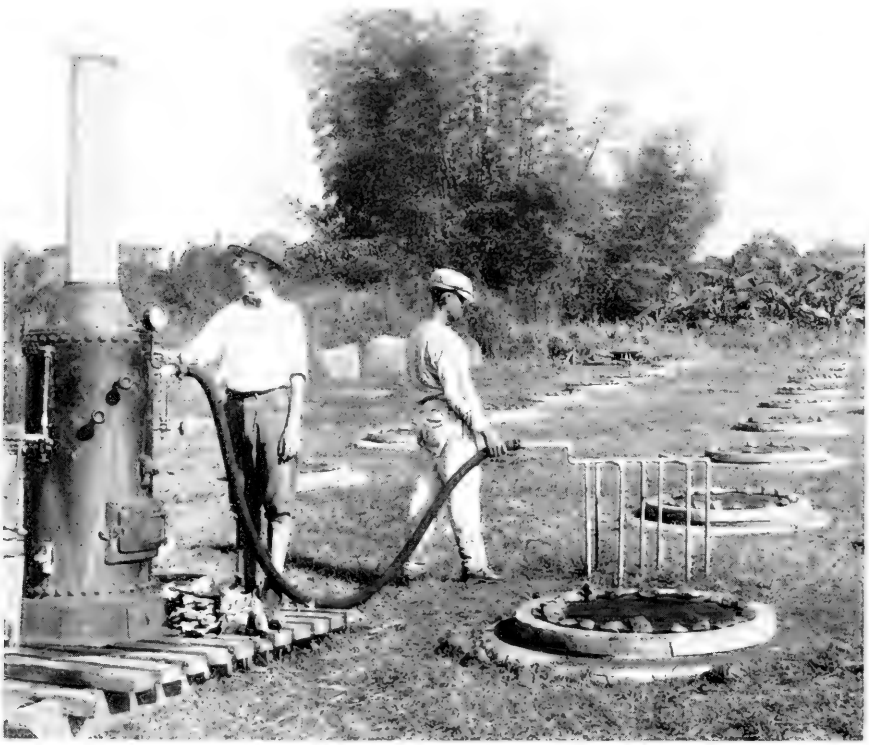

FIG. 3. APPARATUS USED FOR STERILIZING SOIL WITH STEAM

points farthest removed from the pipes had attained a temperature of 120°C. The soil was then covered with discs of tar paper soaked in a strong solution of carbolineum, and the rim was smeared with "tanglefoot" to prevent contamination by insects. It was amply proved by plating out soil from various depths that the soil was actually sterile. When sterilization of the twenty tiles had been completed, the soil in ten of them was inoculated with sub-cultures from a single spore strain of the organism, by emptying the contents of a culture growing in plain corn meal in 1000 cc. Erlenmeyer flasks into each, and stirring in well with

sterile glass rods. All of the thirty tiles, including the ten untreated ones were now planted with healthy bulbs obtained from Naguabo, Porto Rico, a disease free region. The bulbs were first examined for evidence of disease. None was found, but to guard against extraneous infection, they were all treated ten minutes in 1 to 1000 $HgCl_2$ and then rinsed in sterile water.

They were planted by cutting round holes in the middle of the tar paper discs, after which fine mesh wire cages 3 feet high were placed over the tiles. These cages rested on the "collars" of the tiles and fitted closely against the outer part of the projecting rims. The plants were watered with sterile water for several weeks, after which the rains set in, and there was no further need of supplying moisture in this way. In a month it was necessary to remove the cages, due to the growth of the plants. They had served their main purpose, which was to aid in keeping out insects and small animals and thus prevent possible contaminations by other organisms long enough to insure unrestricted growth of the Fusarium in the soil, and also to aid in preventing the possible introduction of the pathogene into the uninoculated tiles in the same way. The tar paper discs, "tanglefoot" and wire mesh cages were merely extra precautions taken to insure the success of the experiment at a time when little was known of the life history of the organism. If without them the inoculated tiles had produced diseased plants and the uninoculated ones, uniformly healthy plants, they could reasonably be regarded as superfluous.

The results of this experiment were convincing. Several of the young plants growing in inoculated soil were severely stunted almost from the start. Two of them died before they were 1 foot tall, but owing to their extremely slow growth, this was a matter of about two months. The balance of the plants in the inoculated row grew rather rapidly at first and at the end of two months some of them appeared to be in as good condition as the control plants growing in sterile soil and also the controls in untreated soil. From that time on, however, a remarkable difference in development took place. Eight months from the time of planting all of the plants in inoculated soil showed unmistakable signs of the disease. Practically every condition mentioned under "Symptoms" was present in one or more of the plants, including, dwarfing, yellowing and wilting of the leaves and splitting of the pseudo-stem (plate XXVI, fig. 2). All of the check plants were healthy appearing and had made a rapid, vigorous growth. The difference between the two rows of plants is shown conspicuously in the accompanying illustration (plate XXX). The check plants, both those growing in sterilized and unsterilized soil, averaged more than ten times as large (by weight) as those grown in inoculated

soil. Dissection of the inoculated plants revealed the typical internal signs of the disease. A fungus was readily isolated from the diseased plants, which when grown in pure culture proved to be identical in cultural characters with the one used for inoculation. The internal tissues of check plants cut down for examination showed absolutely no sign of disease.

Some of the check plants were allowed to grow unmolested to determine if growing in the confined space of the tiles would have any influence in limiting their development. These plants all attained normal size and bore excellent bunches of fruit. Koch's rules of proof were fully complied with in this experiment, and there no longer remains any doubt as to the cause of the disease.

During the course of these investigations it was determined to find out if the organism could attack some of the genera nearly related to Musa, and also some of the plants which are subject to wilt caused by organisms morphologically indistinguishable from *Fusarium cubense*.

Accordingly, plants of the genera Ravanella (traveller's palm), Heliconia (parrots tongue), Strelitzea, and Canna were copiously inoculated with pure cultures of *Fusarium cubense* below the surface of the ground. No infection resulted from any of the inoculations. Later cotton plants (*Gossypium hirsutum*) were inoculated in various ways, with more interesting results. The fungus inoculum was proved to be toxic to cotton but typical wilt symptoms were not produced, nor was there a normal invasion of the vessels. Two methods of inoculation were used, inoculation of the soil, and direct inoculation of the plant tissues. In the first experiment fifteen 6-inch pots of light soil were sterilized in the autoclave by heating three hours at 16 pounds pressure. The soil in twelve of the pots was then very heavily inoculated by emptying the contents of a 500 cc. Erlenmeyer flask containing a two-weeks old culture of *Fusarium* sp. growing in plain corn meal into each pot. The cultures were thoroughly stirred into the soil with a sterile glass rod. Three pots were so inoculated with *F. vasinfectum*, and the balance were inoculated with *F. cubense*, three each from Porto Rico, Cuba and Costa Rica. Cotton seeds, that had been treated for seven hours in calcium hypochlorite (32) were then planted in all of the pots. The very interesting result was that in two weeks from the time of planting, all seedlings growing in the inoculated soil were very much stunted and had curled and distorted leaves, while the check plants showed no such symptoms and were fully three times as tall (text fig. 4). When dug and examined, the roots of plants in inoculated soil were found to be discolored and injured, some of them partly rotted. The vessels in the stem were discolored but hand sections of fresh material did not disclose the presence of any fungus. White

mycelium growing on the surface of rotted roots was proved, upon cultivation to be the Fusarium used for inoculum.

Cotton seedlings three weeks old were next inoculated with macroconidia by making longitudinal slits in the hypocotyl with a sharp scalpel and inserting the inoculum with a platinum needle. The same number of plants and the same strains of Fusarium were used for this experiment as for the preceding. The results were not so uniform, still they were convincing. Where an abundance of inoculum was used the plants wilted and died in from one to two days (text fig. 5). The stems above and to a lesser extent below the wounds became discolored and finally rotted. Where a small amount of inoculum was used the plants were undeniably injured, as was made evident by a retarded development, but they seemed to recover and resumed growth. The control plants,

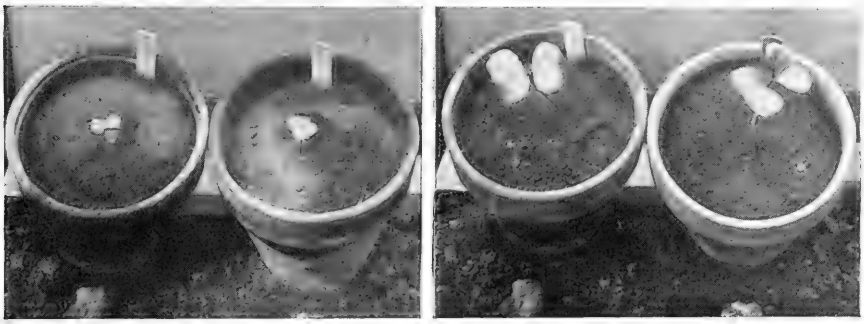

FIG. 4. COTTON GROWING IN SOIL INOCULATED WITH FUSARIUM CUBENSE (left) COTTON GROWING IN STERILIZED SOIL (right)

which had been wounded in exactly the same manner with a sterile scalpel were all half again as tall as the largest of the inoculated plants one week after inoculation. Only one of the plants inoculated with *F. vasinfectum* showed typical symptoms of cotton wilt. The vascular bundles became discolored, and the organism was reisolated from a leaf petiole. Fungus hyphae were determined to be present in the brown, injured petioles of plants copiously inoculated with *F. cubense* also.

It is to be regretted that the results of this experiment were not more decisive. The writer believes, however, that certain conclusions may be drawn from this evidence, when it is supplemented with the results obtained (p. 365) in the experiments on the effect of by-products of the fungus on plants selected at random from the vegetable kingdom. The fact that pathologic symptoms were produced when the banana organism was copiously inoculated into cotton plants is not complete proof that the organism is *F. vasinfectum*.

There are good grounds for assuming that the fungus excretes substances that are poisonous to many plants. It would also appear that the roots of the cotton plant do not exercise any selective absorption with reference to these excreted substances. Where an abundance of inoculum is inserted into the plant tissue through a wound, it seems to have the same toxic effect. The extent of the injury varies directly with the amount of inoculum used.

FIG. 5. COTTON PLANT AT THE RIGHT INOCULATED COPIOUSLY AT THE HYPOCOTYL WITH MACROCONIDIA OF FUSARIUM CUBENSE

Control plant at left. Notice healed wound in control plant

Altogether too little is known concerning the chemistry of the process of penetration of uninjured plant tissues by parasitic fungi. It is assumed from analogy that all such penetrations are brought about by the ability on the part of the fungus to excrete enzymes capable of dissolving cellulose, cutin or lignin. If that were the only essential property, so far as we know of the constitution of cell walls, such a fungus ought to be able to attack almost any plant, but we know to the contrary that a

wonderful degree of specialization exists. Natural infection of its special host by a fungus of this type may be brought about irrespective of the amount of inoculum. It has been shown that the pathologic symptoms in cotton induced by *F. cubense* depends on the mass action of a relatively enormous amount of inoculum. It is believed that the injury to buckwheat and beans was brought about by the same agency. It is proposed, therefore, to consider the two organisms, *F. cubense* and *F. vasinfectum* as distinct, at least until more convincing evidence is brought forward to the contrary.

In conclusion, it appears that this aggressive parasite is known to attack only four (possibly one or two more) of the hundreds of varieties of bananas. In view of this apparent high degree of specialization, it would not seem reasonable to look for natural infection of a plant phylogenetically so far removed as cotton.

d. Names and synonymy

Essed (13) in 1911 as a result of his studies on the disease calls the pathogene *Ustilaginoidella musaeperda.* He figures macroconidia, microconidia and chlamydospores of the Fusarium type, together with an assortment of ascomycetes and even phycomycetes, all of which he calls the same organism. There is some doubt that the Fusarium he figures is the pathogene, since he shows a preponderance of 4-septate macroconidia.

Drost (10) in 1912 designates the pathogene as *Leptospora musæ.* His illustrations show macroconidia and microconidia of a Fusarium, supposedly the imperfect stage of an ascomycete which is also figured. He, as well as Essed, evidently worked with mixed cultures, but since he obtained infection by inoculation with the mixture, probably the pathogene was present.

Dr. Erwin F. Smith (28) in 1910 obtained pure cultures of a Fusarium from wilted bananas sent from Cuba. He demonstrated the ability of the fungus to grow in the xylem of bananas in a greenhouse at Washington, D. C. No technical description of the organism was given, and no comparison with other Fusaria was made, but on the strength of its location in the banana tissue and its evident ability to cause the disease, he considered it a new species and named it *F. cubense.*

This name, then has priority over any other published name, and there is assurance that it is the parasite in question.

The organism is a typical member of the genus Fusarium. It belongs with the group of vascular parasites placed in the section Elegans, provisionally erected by Wollenwebber (33) to facilitate the division of the genus Fusarium into more or less natural groups. Its very close relation-

ship to *F. vasinfectum* has been noted by the writer (6). In common with *F. vasinfectum*, strains of *F. cubense* have the peculiarity of possessing or lacking an agreeable aromatic odor, similar to that of some of the aldehydes of the aliphatic series when grown on steamed rice and certain liquid media. No culture from Porto Rico has been observed to have this odor. It is present or absent in cultures from Cuba. All cultures that the writer obtained in Panama, Costa Rica and Jamaica generated the substance causing this odor. This difference is sharply demarked, and seems sufficient to justify the erection of a new variety.

Fusarium cubense

Fusarium cubense, E. F. Smith, 1910, in Science n.s., v. 31, no. 802, p. 754, 755.

Ustilaginoidella musaeperda, Essed, 1911, in Ann. Bot. **25**, pp. 343–361.

Leptospora musae, Drost, 1912, in Bul. 26, Dept. van der Landbouw Suriname.

Sporodochia on leaf stalks and blades, separate, arising from pseudoparenchymatous substratum in substomatal cavities, hyaline; conidiophores septate, projecting through stomatal apertures, hyaline, verticillately branched, about 70 μ long and 4 μ in diameter, apical end tapering gradually to a point; branches in whorls of 3, continuous, arising from upper end of cells of the conidiophore, apical ends tapering abruptly; microconidia hyaline, oval or elongate, continuous, 1- or 2-septate, 5 to 7 μ by 2.5 to 3 μ; macroconidia of the Elegans type, 3- to 5-septate, sickle-shaped, pedicillate at the base, more than 95% of 3-septate macroconidia present, 22 to 36 μ by 4 to 5 μ, a few 4- and 5-septate conidia, born at apical ends of conidiophores and lateral branches.

In pure culture on potato plugs, indigo blue sclerotia, irregular, nodule like, 1 to 4 mm. in diameter, produced, in 12+days. Pionnotes produced on *Melilotus alba* stems, conidia salmon-colored in mass. On steamed rice grains a pink or pinkish salmon color imparted to the substratum at first, later becoming blotched or streaked with red, and finally tinged with vinaceous purple or wholly blue. Strong aromatic odor on this medium. Chlamydospores ellipsoidal to globose, terminal, intercalary or conidial, simple, paired or catenulate, when unicellular 5.5 to 6 μ by 6 to 7 μ. Vascular parasite, causing wilt of *Musa sapientum*.

Fusarium cubense var. **inodoratum** n. v.

Differs from *F. cubense* by the absence of odor on steamed rice, etc. Vascular parasite, causing wilt of *Musa sapientum*.

e. Life history

There are two, so to speak, distinct life histories of *F. cubense*, in one of which the parasite may be constantly associated with the host for many years. In the other, there is a definite alternation of pathogenesis and saprogenesis.

The first of these owes its existence to the method of propagating the banana, and the ability of diseased rhizomes to produce suckers which are only slightly infected. These latter are sometimes able to make a strong growth, and in turn produce suckers before the disease terminates in death. The slightly affected suckers may remain attached to the parent stool, or may be cut away and planted elsewhere. Owing to the carelessness and ignorance of man, this practice has resulted in a wide distribution of the disease. If there were no other method of spreading the disease, it would be restricted to the progeny of originally diseased plants. Unfortunately, however, nature has provided for an almost unlimited dissemination of the pathogene to new localities under the proper conditions. It will not be necessary to discuss the details of the growth of the parasite in the host, where it is constantly associated with the latter, since the process is essentially the same as in the other method. It may be mentioned merely that the vascular systems of parent bulb and sucker are of course continuous, so there is no obstruction to invasion of the latter from the former. The second type of life history will be discussed in detail.

1. Source of inoculum. The inoculum consists of macroconidia and microconidia (plate XXXI, fig. 1) produced by minute sporodochia (plate XXXIV, fig. 2) which are born on both surfaces of the leaf blade, on the leaf stalk or even on the leaf bases which form the pseudostem. The sporodochia are most numerous in the "axils of the leaves," where the leaf stalk diverges from the sheathing leaf base. Conidia apparently are produced at any time of the year, the governing factors being the stage of the disease and high atmospheric humidity, or abundant rainfall.

2. Dissemination. They are loosely attached to the conidiophores, not being held by a gelatinous matrix or any other provision to prevent dissemination when dry, so that they are easily dislodged by the wind, and carried on slight currents of air. Sterile agar plates, exposed for one-half hour beneath diseased banana plants and between the rows in the plantation at Mayaguez, Porto Rico, have yielded colonies of *F. cubense*, the number of colonies per plates varying from 1 to 30.

It is not known just how far these conidia may be carried by the wind in a viable condition. They are extremely small and light. Judging from the results of experiments (22) with uredospores of *Cronartium*

ribicola in which it was found theoretically possible for them to be carried many miles, it may be assumed that the limiting factor in the dissemination of these conidia by the wind is really their desiccation deathpoint.

In the tropical downpours which are so common in many banana producing countries, it frequently happens that the water accumulates on the surface of the ground so rapidly that it is not immediately absorbed, but runs in sheets or streams for considerable distances. There can be no doubt that this affords a ready means for the dispersal of spores for short distances.

In the main the conidia so produced suffer one of two fates, they are either carried to the infection courts of fresh victims, or they are deposited on other plants or on the soil. A preponderating majority of the conidia produced suffered the latter fate. It has been abundantly proved that the fungus may remain alive in the soil in some form or another for long periods of time (6). Samples of soil taken under aseptic conditions from banana plantations and adjoining fields, and plated out by the loop dilution method show that the organism is present therein in enormous numbers. For these determinations, it is preferable to use a specially prepared medium. It should be acid enough to inhibit the growth of bacteria and should contain some form of starch so that the organism may be quickly recognized by its characteristic color reaction.

On account of the number of colonies produced on the agar plates (many thousands per gram of soil), it is thought that the organism is present in the soil as spores of some kind. Slight success has attended the direct microscopic examination of soil. Soil samples were taken in the same manner as for plating out, and were placed in small vials of sterile distilled water, about 1 gram of soil to 10 cc. of water. This was shaken up thoroughly, and when the larger particles had settled, a loopful of the suspension was placed on a clean cover slip. It was allowed to dry in the air, and was then killed and fixed by passing rapidly through a flame. The preparations were stained one minute in a 2 per cent solution of Bismark brown in 70 per cent alcohol, and mounted in Canada balsam. The chief difficulty in this method is the extreme dilution necessary. It is estimated that with the maximum number of fungus bodies present according to plate count, in whatever form they might be, many such preparations could be made that would not contain a single body. They would, however, contain thousands of fragments of organic matter, minute bits of sand, crystals, etc. Confusion due to the latter two is largely eliminated by the fact that they do not take the stain. Positive identification of biologic forms in such preparations may be said to increase in a geometric progression with the number of such forms present.

The writer searched such preparations diligently for a whole day and was rewarded by finding two Fusarium macrospores, one of which had an intercalary chlamydospore, several microspores and numerous bodies that might have been detached chlamydospores. No vegetative structures were seen whatsoever. These preparations were saved. There can be no doubt as to the identity of the macrospores, that is, they are unquestionably forms of a Fusarium. The other findings would seem to indicate that chlamydospores may be formed. The absence of vegetative structures does not prove that the organism cannot grow in the soil. Laboratory experiments suggest strongly that it can. Large test tubes containing sterile, moist, clay loam were inoculated at the top. After one month the bottom of the tube was broken and a fragment of the soil plated out, with the result that the organism was recovered at this point. Later U-tubes of soil were inoculated at the top of one arm, and the organism was subsequently recovered from the top of the other arm.

The organism then is capable of growing in the soil. In what form it may be is still somewhat obscure, but it is evident that if healthy bulbs are planted in such soil, they probably can become infected. This leads to consideration of another method of distribution of the fungus. Mud, carried on the feet of men and animals, or on the wheels of vehicles would serve as a medium for dissemination of the fungus for great distances.

Infected banana leaves are frequently found among the "trash" used for protecting bunches when they are packed in cars for hauling to the steamers. This trash is often carelessly thrown from the train at various points along the route. It may be carried for great distances on the floor of cars and probably has aided in distributing the fungus to new regions.

Insects may act as carriers, but there is no definite information on this point, and in the opinion of the writer it is of little importance.

3. Infection courts. It has been noted (p. 355) that the disease progresses from below the surface of the ground upward, originating either at the large wound on the rhizome caused by separating the sucker from the parent stool, or at the side of the bulb where a root is given off. Attempts to infect any of the above ground parts, either by spraying spores on the unwounded surface, or by inserting the inoculum into wounds have never given rise to the typical disease. Limited local growth of mycelium in the vessels may follow inoculating into the tissues of pseudostem, stem or leaf, but the method is not uniformly successful, and has never been known to cause death or even serious symptoms. That the unwounded young roots and wounded rhizome are the natural infection courts was proved by the following experiments.

A young healthy plant was selected and the roots carefully exposed by washing the soil away with a jet of water. Drops of a thin spore sus-

pension were placed on the uninjured thread-like roots about six or eight inches from the bulb. Sterile moist cotton was wrapped about the point, and the soil replaced. In twelve hours the root was removed and the inoculated portion killed and fixed in chrom-acetic acid.

Similarly the rhizome was partly exposed and a cut made in the stele with a sharp razor. The flat cut surface was inoculated in the same way, protected with cotton, and the soil replaced. After twelve hours, cubes of the stelar tissue were removed from the inoculated surface, in such a way that the latter were included as one face of the cube. These were also killed and fixed in the chrom-acetic acid solution. All of the tissues were then washed, dehydrated, infiltrated with paraffin in the usual way, imbedded, and radial, transverse tangential, and serial sections, were cut with the microtome. The sections were stained twelve hours in safranin and three minutes in Delafield's hæmatoxylin, and mounted in balsam. Microscopic examination of these slides showed that in the case of the cut stelar tissue, the mycelial threads had in twelve hours penetrated the cut ends of vessels, and the parenchymatous starch containing cells in great abundance. In some cases the fungus was demonstrated by transverse sections to have passed through eleven cell walls. The average depth of penetration was from eight to ten cells from the cut surface. Beyond this point there was no invasion and the tissue was normal.

In the case of the thread like roots, there was no evidence of penetration whatever. The spores had germinated and grown among the root hairs, but were not seen to penetrate them, or the epidermal cells of the root at all. The roots used in this case were some distance from the bulb and the cells were mature, i.e., the tip of the root had not been used for inoculation. It was determined to repeat the experiment, using young roots to see what effect the fungus might have on meristematic root tissue and young cells just back of the root cap. Several of the large fleshy roots, just emerging from the cortex of the rhizome were selected for this purpose. They were not more than $\frac{1}{2}$ inch long at the time, and were possibly $\frac{3}{8}$ of an inch in diameter at the base. They were exposed and inoculated without injuring them, in the same manner as previously described for the thread like roots. The same culture was used as in the former inoculations. After twelve hours they were removed, killed, fixed, and stained, serial sections prepared as before described. Upon microscopic examination a quite different condition was found to exist (plate XXXI, fig. 2). The epidermal cells and cortex had been deeply penetrated. The root hairs (fragments of which are shown at the surface in the photomicrograph) were not attacked, and do not serve as a means of entrance for the parasite.

The xylem elements of the young roots were not at all lignified at this stage of development (as indicated by their not taking the red of the safranin stain), and would not offer any resistance to invasion by the fungus. Proof, then, that the young fleshy roots, and injured stelar tissue act as infection courts is considered to be amply furnished by these experiments.

4. Type of penetration. Penetration by both macroconidia and microconidia is accomplished directly by means of a germ tube. The germ tube may arise from any cell of the macroconidium. It is quite thick, at least equal in diameter to the conidium from which it arises. This is in great contrast to the germ-tubes of conidia germinating in water, where they are very attenuated and thread-like (plate XXXII, fig. 1). It is not known by what means the tip of the germ tube penetrates the outer cell wall of the epidermal cells, but it is probably accomplished by the excretion of some dissolving enzyme. These cell walls in the young root tip are not especially thick, and do not seem to be cutinized or cuticularized. With the stains used, they did not react differently than the other cell walls of the cortical tissue.

5. Growth of the parasite in the host. Immediately upon penetration of the epidermal cells, a food relationship is established with the host, and the germ tube becomes rapidly growing mycelium. There are no special absorption organs. The contents of the host cells is evidently absorbed at any point in the undifferentiated cell walls of the parasite. The mycelium is septate and profusely branched from the start (plate XXXI, fig. 2). Growth is intracellular and the cell walls seem to offer little resistance to the passage of the hyphae. When the xylem elements are reached, the fungus grows in the lumen of the trachae, proceeding towards the stele. The most abundant vegetable growth found anywhere in the vascular tissues of the host occurs in the root vessels, close to the point of infection. Evidently growth proceeds more rapidly in the vessels than elsewhere, for a cross section of the root where it passes through the fleshy cortex of the rhizome, will reveal a still abundant growth of mycelium in the lumen of the vessels (plate XXXII, fig. 2), but none can be detected in other cells of the vascular bundle. The mycelium now continues its growth through the continuous system of vessels, entering the stele (plate XXXIII, fig. 1), thence passing again through the cortex at the upper part of the rhizome, where the vascular bundles form the leaf-traces, and so on up into the leaves, but apparently always being confined to the vessels. Soon after entering the young xylem elements in the root, when growth has proceeded a little distance towards the rhizome, the fungus finds itself in the older, lignified tracheae. Probably there would be more difficulty in penetrating the walls of these old lignified vessels, if indeed it were not impossible, so the

fungus follows the line of least resistance upward through the lumen, unimpeded by cross walls of any kind. At least it is a fact, that once the fungus enters the vessels of the root, it does not escape from the vessels until fructification takes place high up in the leaves. It has been remarked that the mycelium in the vessels of the pseudostem is very scant (plate XXXIII, fig. 2). It has never been observed to be abundant in vessels anywhere excepting in the infected root close to the point of inoculation. A logical explanation of this is to be found in the meager supply of organic food in the vessels. Studies on the nutrition of heterotrophic plants show us that a luxuriant growth is not to be expected in the absence of an appreciable quantity of organic food. Where would this be obtained in the vessels? Unless the roots have absorbed some organic substance in solution (20) which may be possible in some plants, the available food would consist of certain salts in solution, and possibly some material obtained by the partial digestion of the spiral or reticulated secondary thickening of the vessels. There is no known abundant source of food in this situation, and it is not surprising that the vegetative growth of the fungus is not luxuriant. This paucity of food may account for the production of microconidia in the vessels of the pseudostem, noted by Dr. Erwin F. Smith in 1910 (28).

It is known that with many fungi, fructification takes place more readily after the medium has become impoverished.

The scant mycelium extends in the late stages of the disease to the leaf stalk and even to the midrib of the leaf. By the time that this has occurred, the leaf has exhibited external symptoms of disease. It has become yellow, and possibly wilted, so that it hangs limply by the side of the pseudostem. At this point, probably due to the weakened condition of the host, the fungus escapes from the vessels, and is found in the sieve tubes, and in fact in all of the parenchymatous cells of the leaf stalk. It is particularly abundant in the epidermal cells and subepidermal cells (plate XXXIV, fig. 1). The mycelium is here very abundant branching and intracellular. The subsequent development of sporodochia and the production of conidia have been discussed under "morphology of the causal organism" and need not be repeated here.

7. ECOLOGY

The severity as well as the spread of banana wilt are strikingly correlated with certain well defined weather conditions. Wet weather, especially if it is characterized by dashing rains with movement of surface water, is apparently a contributing factor of considerable importance in the dissemination of the pathogene (cf. p. 376). This assumption is based

on field observations in Porto Rico. There, it may readily be seen that shortly after the rainy season has set in, there is a decided increase in the number of apparently new cases, and an unmistakably more rapid progress of the disease in plants already infected. The first of these phenomena may be accounted for in either or both of two ways. Laboratory experiments (p. 375) indicate that the conidia are very subject to injury by desiccation. During the dry season the surface of the soil is very hot and dry. The is little likelihood that conidia deposited thereon would survive. In addition to that fact, a certain amount of atmospheric humidity seems essential to the development of sporodochia, at least they are more abundantly produced when a high degree of humidity prevails. The other explanation is that the young, fleshy roots, which are known to be especially susceptible infection courts, are not developed during the dry season, but push out from the rhizome in large numbers after the rains have set in. The explanation for the other observed phenomenon, namely, that previously infected plants go down with the disease much more rapidly in wet weather, is more obscure. The answer may be sought in the fact that there is a retarded transpiration and consequently less conduction of water and solutes in the vessels when the soil is dry, so that the toxin assumed to be excreted by the fungus would not be carried upward into the pseudostem and leaves in such large amounts. In wet weather on the contrary, due to the abundance of soil moisture and the hot dry winds that prevail during a part of the day, transpiration would be more active, and conditions providing for a more plentiful upward distribution of the water and its inclusions would prevail.[6]

It has been briefly mentioned that in arid regions, where irrigation is necessary for the successful production of bananas, the disease is unknown. The writer has never seen nor heard of a case of banana wilt in the arid regions of southern Porto Rico, southern Jamaica or northern Colombia, with the exception of one plant observed in St. Catherine Parish, Jamaica, which was evidently accidentally inoculated with material brought in for experimental purposes. In such regions the intense heat and dryness of the surface of the soil would make the survival of wind-born conidia impossible. In southern Jamaica, the soil is so hot and dry that planters invariably set the bulbs with about 1 foot of the old pseudostem still attached, so that the succulent latter portion when it rots will furnish moisture for the young "peeper" and prevent its death by "boiling" as it is termed. "Baking" would be a more descriptive term. Attempts to isolate the organism from such soils failed, but it was readily isolated

[6] It was not proved experimentally that there is actually more transpiration in the wet than in the dry season.

from soil in the vicinity of a diseased plant in northern Jamaica where rains are frequent. It is believed that the disease need never be feared in these arid regions. It is probable that in times past infected bulbs must have been imported into such areas, but if they were, it is evident that under the prevailing conditions the plants either outgrew the disease or were killed, and the suckers were not used for propagation.

In regions where there are no well demarked wet and dry seasons, that is, where the rain is more or less evenly distributed throughout the year, optimum conditions for dissemination of the fungus exist. This is attested by the virulence of the disease in the banana districts of Panama and Costa Rica. Even there one finds an occasional drought, but no well defined annual dry season. In Nicaragua, Guatemala, and Honduras, conditions are about the same, so it is to be expected that a repetition of the history of the malady in Panama will take place in those countries unless extraordinary steps are taken to prevent it.

In Surinam, where the annual precipitation is very heavy, and the rains are distributed over practically the whole year, the disease spread over the entire country in the short space of four years. In such regions it is not only highly infectious, but extremely virulent and aggressive. Attacked plants succumb to the disease very quickly, whereas in dryer regions the plants may survive for many months, sometimes nearly a year after becoming infected.

8. CONTROL

Introduction

A scrutiny of the life history of the pathogene will convince one that its elimination as a source of injury to the banana plantations in the countries where conditions are favorable to it, cannot be based on an attempt to eradicate the parasite. It is true that a rational system of sanitation, based on the findings of this paper, would aid in suppressing the disease somewhat, and if universally practiced for some years might materially reduce the amount of loss. Any direct method of attack, however, such as protecting the plants by the application of fungicides or eradication of the parasite in the soil by any method now in use is obviously out of the question. Aside from the fact that fungicides which migh be used to treat the bulbs before planting would soon become ineffective, the very susceptible young fleshy roots, as they pushed out into the soil would be absolutely unprotected. Sterilization of the soil is not practicable considering the present market price of bananas. Selection of disease-free bulbs is of value only where they are to be planted in soil which is not already infested. It is manifest that in view of the different

climatic and soil conditions and other factors, the question of control measures presents separate and distinct problems in the several banana-growing regions. Experiments on the control of banana wilt in badly infested areas at the Porto Rico Agricultural Experiment Station have up to the present led to no very definite conclusions. It is considered of some value, however, to briefly mention a few of the methods of attack, even though for the most part they gave negative results. Control measures for this disease, as for other diseases of plants would naturally group themselves under one or more of four headings, namely exclusion, protection, eradication and immunization.[7]

1. Exclusion (quarantine)

Exclusion of diseased plants, plant parts or other infected material by legislation would be of value only: (1) in countries where the disease is not present but in which conditions are such as favor the disease, (2) in similar regions where the disease is not yet firmly established, or (3) where it is partly held in check by climatic conditions.

Honduras and parts of Guatemala would be included under the first of these divisions. It is probable that a few isolated cases of the disease exist even there. Under the second division would be the balance of Guatemala and Nicaragua. The last division would include Jamaica, Porto Rico and perhaps Cuba. Quarantine in connection with this disease may be general, local, or both, depending on various factors. It is believed that in Honduras strictly enforced legislation providing for the exclusion of banana plants or plant parts which might be sent from any other banana region with the possible exception of the arid north coast of Columbia would be a practical and profitable measure. In addition, regulatory laws in regard to the importation of tools, machinery, etc., formerly used in the cultivation or handling of bananas in other regions, providing for their sterilization by fungicides is not considered an extreme precaution. Such laws already exist in Jamaica. Orders have been issued by the Governor of Jamaica in accordance with the laws, calculated to prevent the introduction into the island of the inoculum in any form. Furthermore, in Jamaica, this disease has been declared notifiable, that is, it is required by law that any person occupying land on which plants infected with banana wilt exist, must give notice of the same to the Director of Agriculture, who then directs that the treatment prescribed by law be carried out. This treatment consists of the destruction by fire of the diseased banana plant or plants, and all other plants surrounding them

[7] These terms are used by Prof. H. H. Whetzel, head of the Department of Plant Pathology, Cornell University, in his lectures on plant disease control.

within a distance of one chain (22 yards), after which the infected area is fenced in and a local quarantine established for one year or until such time as it may be lifted at the discretion of the Director of Agriculture. Several hundred cases were reported and treated in 1915 and 1916 but less than a score were found in 1917, two years after the law became effective. These far-sighted laws were passed at a time when practically nothing was known of the life history of the pathogene. Investigation of the latter shows that they were amply justified. Certain features of the law could doubtless be improved upon, but the leveling and destruction of all affected plants, which means that they could not be used for propagation, or serve as a source of wind- or rain-born conidia, and the exclusion of traffic by man and animals through the infected area, has unquestionably served in a large measure to prevent the spread of the disease.

It is believed that the progress of the disease would be greatly retarded in Honduras or other countries where only isolated cases occur, if similar steps were taken. It is thought also, that the same measures would be practical in regions where the disease has gained more of a foothold, but where its progress is somewhat retarded owing to the fact that optimum conditions for dissemination of the pathogene are lacking. This of course has been abundantly proved for Jamaica conditions. Porto Rico and Cuba are included in the regions of this character. It is realized that this procedure does not constitute absolute control of the disease. Even in places where it is applicable, it appeals to the writer merely as a temporary measure calculated to alleviate the situation, to be used pending the time when banana wilt may be eliminated by some other method.

2. Protection

Protection is defined as the interposition of some effective barrier between the susceptible part of the host and the inoculum of the pathogene. It has been found that the susceptible parts of the host are below the surface of the ground. This means that the necessary renewal of any substance toxic to the fungus would be impossible. Additional applications of such substances are necessary to provide protection for the increased new areas brought about by growth, as well as for the purpose of renewing the inhibiting substance on old mature parts as it gradually weakens due to washing and leaching away. Any method of attack, similar in principle to the spraying of above ground parts is impracticable. That the application of such a principle is unsound is apparent to any one now that we have positive knowledge of the life history of the organism, nevertheless cases have come under the observation of the writer where investigators have attempted to control this disease both by the application of fungi-

cides to the leaves and pseudostems and by dipping the bulbs in fungicides previous to planting. The fallacy of attempting control measures before completion of the study of the organism is recognized, yet in the hope of saving time, much useless expenditure of energy and money is often made. The writer was exceedingly mortified by the result of an experiment on the control of this malady, started at a time when evidence seemed to point to dissemination of the pathogene through the medium of the soil. It is here mentioned in support of the above statement. Isolation of the organism from the soil had been accomplished, and field observations on the origin of new cases led to the belief that the fungus spread through the soil by vegetative growth. A field which had been growing sugar cane for the previous three years, and vegetables for some years before it was put into cane was selected for the experiment. A badly diseased plantation of bananas adjoined the field on two sides. Trenches 2 feet deep were excavated in rows 10 feet apart both ways. The plat was about 100 feet from the edge of the banana plantation. Examination of the soil did not reveal the presence of the fungus. Healthy banana bulbs were planted in the center of each 10 foot square made by the intersection of the two parallel series of trenches, the idea being that an effective barrier to the growth of the fungus would be made by removing the top layer of humus containing soil. One after another of the plants fell victim to the disease. One year after the experiment was started 60 per cent of them were affected. We now know that it was perfectly possible for such infections to have resulted from wind borne conidia. Such a method of protection is of no more value than the application of fungicides.

3. Eradication

Practically all of the efforts so far made in attempting to control this disease have been directed along the lines of eradicating the parasite.

The first principle of crop sanitation is to avoid planting infected bulbs which invariably give rise to diseased plants. The necessity for selecting healthy bulbs for planting cannot be too strongly impressed upon banana growers. It is of the utmost importance in recently opened banana land where new plantations are being layed out. The practice should be followed even where the disease has been established, but it must be remembered that when clean bulbs are planted in infected soil, many of them will become diseased. When examining bulbs for evidence of disease, it is essential that the machete be sterilized by fire or by carefully wiping it with a cloth soaked in some fungicidal solution every time a fresh cut is made. Every bulb which has the appearance shown in plate XXIII, fig. 2, in whatever degree the discoloration may be present should be

rejected or destroyed by fire. Only bulbs which look like the one figured in plate XXII, fig. 2, are suitable for planting. These should be kept carefully separated from the diseased bulbs after sorting.

When a diseased individual is detected in the field, it should be immediately rooted out and destroyed since it soon becomes a menace to surrounding plants on account of the production and dispersal of conidia. Where only isolated cases occur, and firewood is available the entire plant should be removed from the ground, cut into thin slices with a machete and burned. On account of the succulent nature of the banana plant this entails much labor, but heedless neglect of such plants is bound to reap a harvest of new cases.

Eradication of the pathogene in the soil by allowing the land to lie fallow, rotation of crops, disinfection of the soil, liming, mulching, flooding and other methods have been tried, but none have yet been found practical.

The longevity of the organism in the soil is not definitely known, but it has been isolated from land which had not been in bananas for five years. Bulbs planted in this field quickly became diseased.

In connection with studies on the etiology of the disease it was found that steam sterilization of the soil was very effective, but it is needless to say that under present conditions this method is impracticable. Disinfection of the soil in small plats by drenching with copper sulphate, carbolineum and formaldehyde was not only unsuccessful, but the expense prohibitive. Other attempts to eradicate or render innocuous the parasite in the soil were worse than useless.

4. Immunization

The successful measures described up to the present are at best only palliative, and are applicable only in restricted areas where the disease has not yet become rampant. It remains for some method of control to be developed whereby the vast areas of fertile banana land in Panama, Costa Rica and Surinam which have been absolutely abandoned owing to the ravages of the banana wilt pathogene may once again be made productive. The writer is convinced that there is only one solution to the problem, and that lies in the development of resistant strains of the desirable varieties of bananas. Attempts have been made to substitute resistant varieties for the ones now on the market, but all of them have had one or more fatal defects which have prevented their meeting the requirements of the market, the shippers or the growers.

Experiments have been started at the Porto Rico Agricultural Experiment Station in the hope of obtaining resistant strains of the Chamaluco banana. The process of selection is necessarily long and tedious

with a crop that requires more than a year to mature. It is too early to make predictions as to the outcome, but the indications are such that a successful termination is awaited. It was observed that in a large plantation of Chamaluco bananas on the experiment station grounds which had been a veritable hotbed of infection for some years, an occasional plant would resist the disease and make a normal growth. These plants produced good bunches of fruit, and gave rise to healthy suckers. Apparently optimum conditions for infection existed. The healthy stools had been surrounded by diseased plants for many generations. The progeny of these healthy plants were removed on June 23, 1916, to a specially prepared field which was artificially inoculated as heavily as possible. Diseased plants of this variety had been cut into small pieces distributed evenly over the surface of the field and worked into the ground. It was thus proposed to give the parasite every chance for infection. The progeny of the stools that survive will be subjected to the same treatment.[8] If they in turn survive, it is believed that they can withstand the disease under any conditions, and they will be propagated and distributed as immune strains.[9] It is strongly recommended that similar experiments be started with commercial varieties in the countries where bananas are grown for export.

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[8] Since these experiments were started Edgerton (12) has outlined a method of obtaining wilt resistant tomatoes by first sterilizing the soil, and then inoculating it heavily with pure cultures of the organism.

[9] In April, 1919, the writer was in Porto Rico and paid a visit to this field. Of the one hundred and five selected plants, sixty had survived the treatment. Suckers from the latter healthy plants were set out in the same field after it had been plowed up and reinoculated in the same manner as above described.

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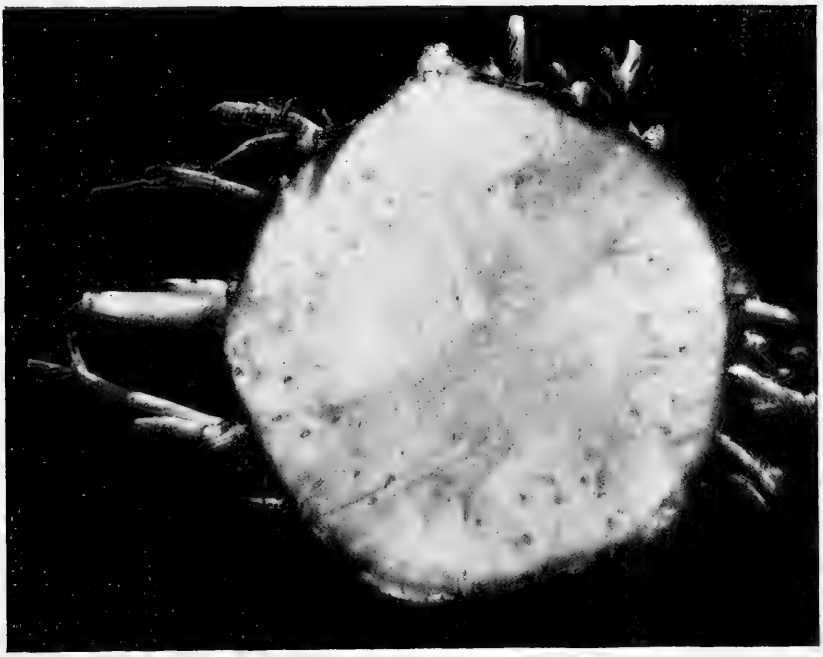
Plate XXII

Fig. 1. Cross section of healthy banana pseudostem.
Fig. 2. Cross section of healthy banana rhizome.

1



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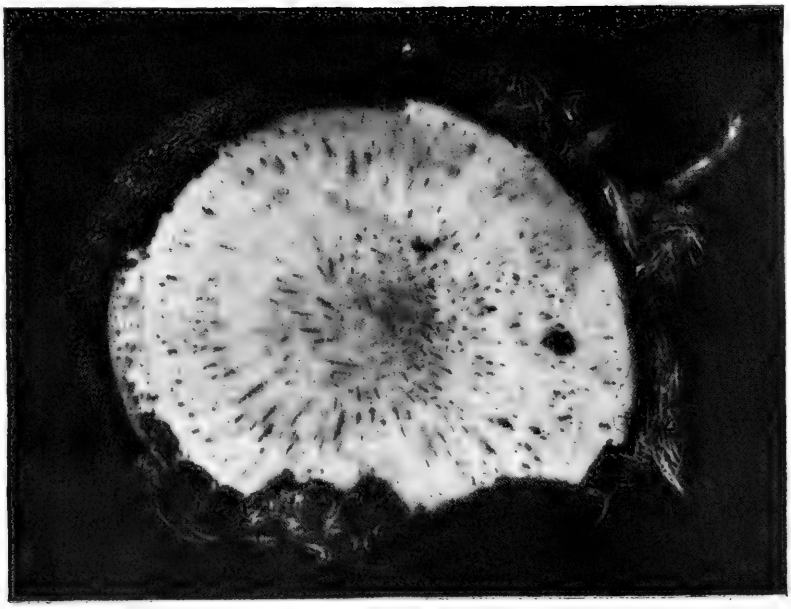
BRANDES: BANANA WILT

PLATE XXIII

FIG. 1. Cross section of diseased banana plant. The plane of the section includes the upper part of the rhizome (at the center) and a few of the sheathing leaf bases which form the pseudostem (at the periphery).

FIG. 2. Cross section of diseased banana rhizome.



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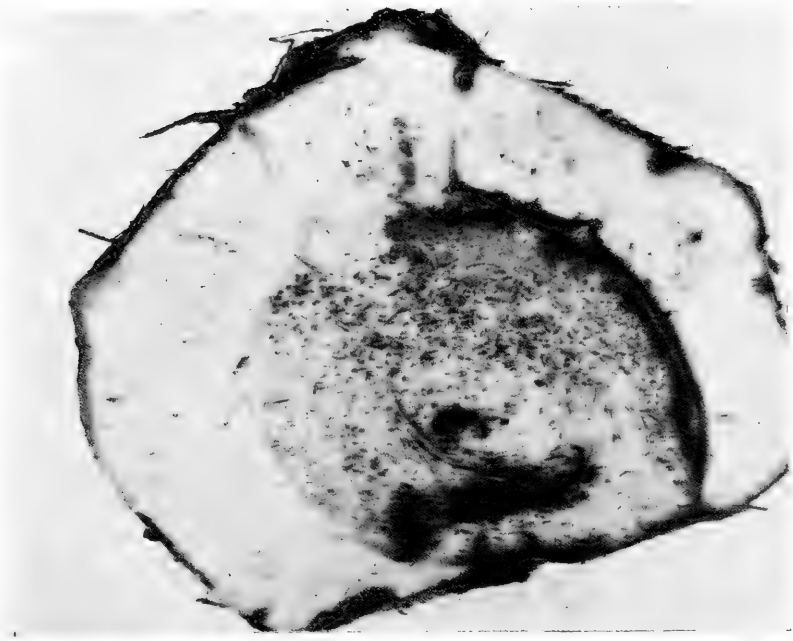

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BRANDES: BANANA WILT

PLATE XXIV

FIG. 1. Banana plant killed by wilt organism after starting to produce a bunch of fruit. Apparently healthy sucker arising from the old stool.

FIG. 2. Last stage of the disease. Plants of the Chamaluco variety in Porto Rico.

1

2

PLATE XXV

FIG. 1. Longitudinal splitting of the pseudostem. Manzana variety in Cuba.

FIG. 2. Longitudinal splitting of the pseudostem. Gros Michel variety in Costa Rica.

1 2

BRANDES: BANANA WILT

PLATE XXVI

FIG. 1. Longitudinal splitting of the pseudostem. Chamaluco variety in Porto Rico.

FIG. 2. Longitudinal splitting of pseudostem produced by artifical inoculation. Chamaluco variety in Porto Rico.

1

2

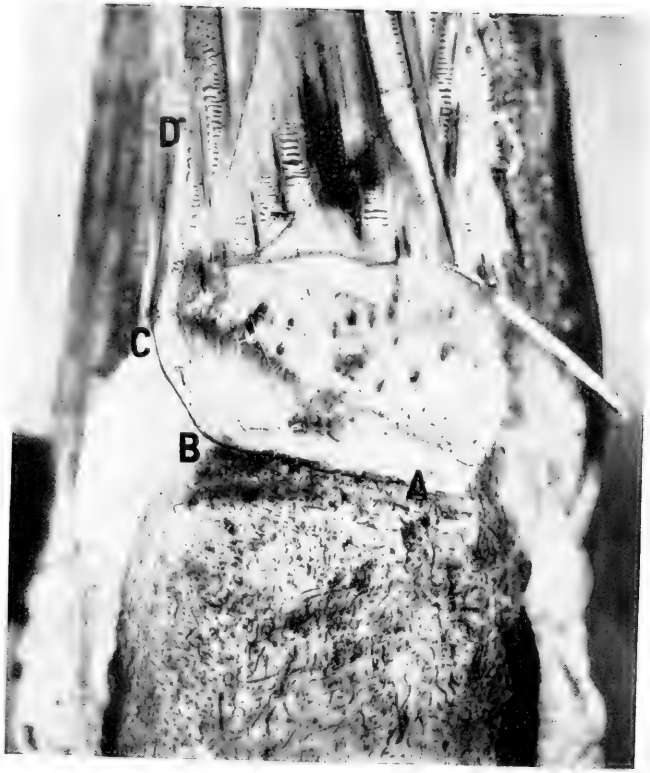
BRANDES: BANANA WILT

Plate XXVII

Fig. 1. Longitudinal section of banana rhizome and pseudostem showing discolored leaf traces in the cortex, and diseased roots passing from the diseased stele across the otherwise healthy cortex and out into the soil.

Fig. 2. Longitudinal section of banana rhizome and pseudostem, showing individual diseased vascular bundle passing from the stele at *A*, through the endodermis at *B*, and traversing the cortex *B* to *C*, thence passing up into the leaf base. Notice that the cortical tissue other than the diseased strand is not invaded, the fungus being confined to the vessels of the vascular bundles.

1



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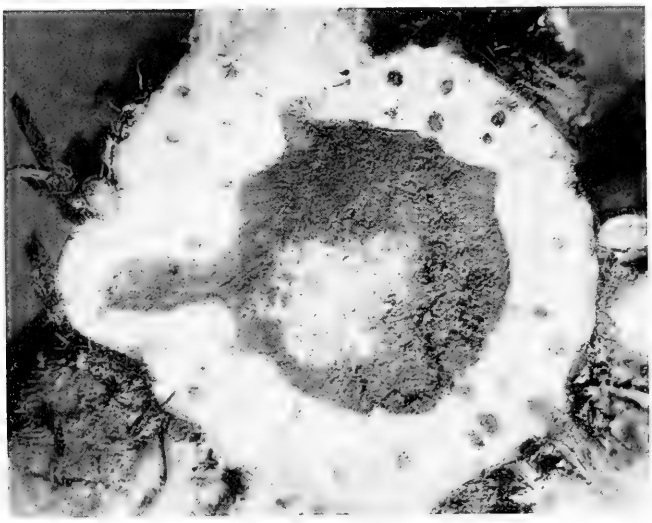
BRANDES: BANANA WILT

Plate XXVIII

Fig. 1. Transverse section of rhizome showing general infection. Gros Michel variety, Costa Rica.

Fig. 2. Transverse section of rhizome showing late stage of the disease. Secondary rots have set in. Notice the still healthy appearance of the cortex. Chamaluco variety, Porto Rico.

1



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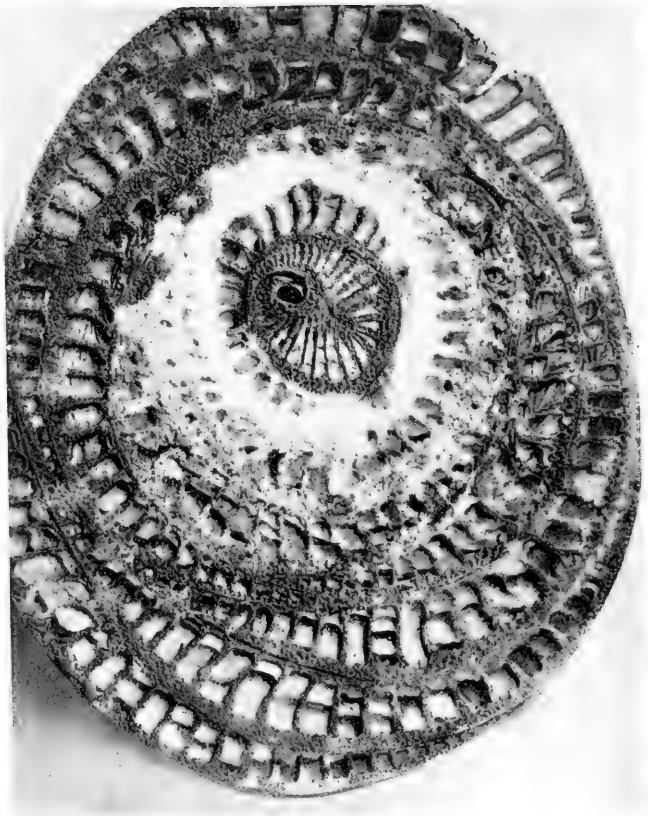
BRANDES: BANANA WILT

Plate XXIX

Fig. 1. Transverse section of pseudostem of Chamaluco variety in Porto Rico Notice freedom from disease of central and outer portions. The diseased portion is represented by the band of white mycelium concentric with the periphery. This piece of tissue was kept in a moist chamber for three days before the photograph was taken.

Fig. 2. Transverse section of rhizome showing local infection of stele through a root. Gros Michel variety, Jamaica.



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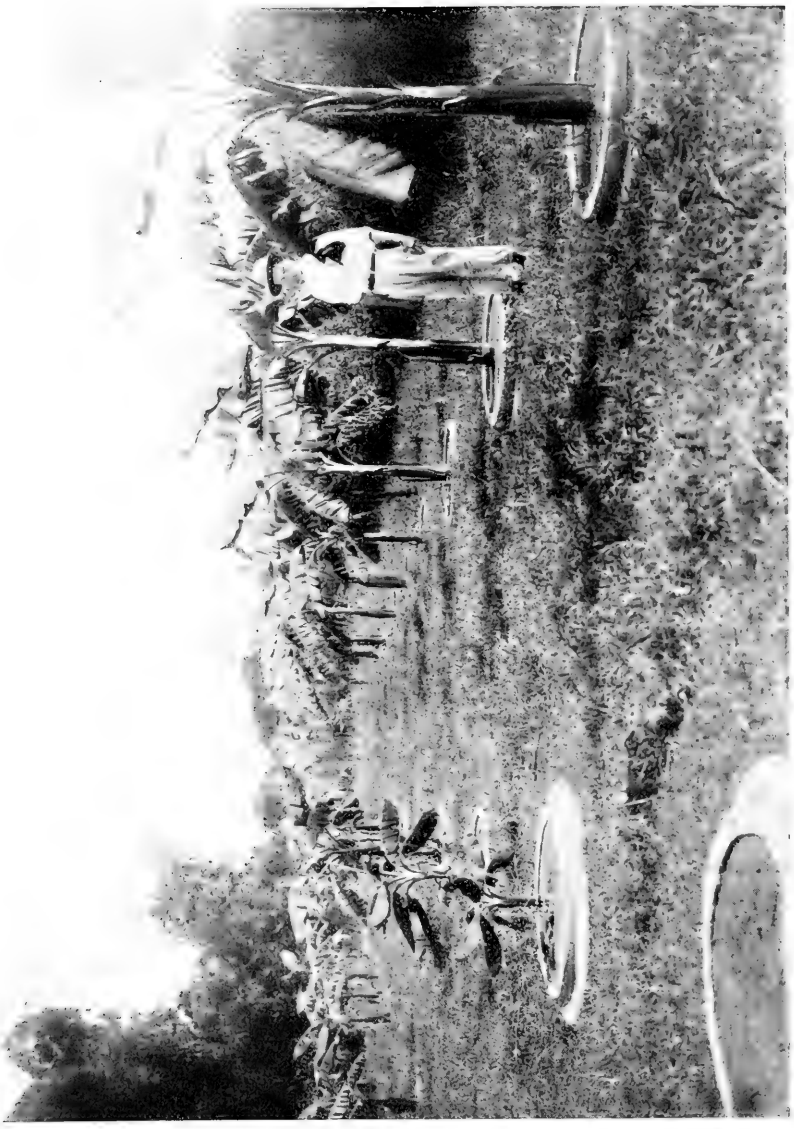
Brandes: Banana Wilt

Plate XXX

Result of experiment on inoculation of soil with pure cultures of *Fusarium cubense*. Row on left inoculated, row on right not inoculated. Time, eight months after planting.



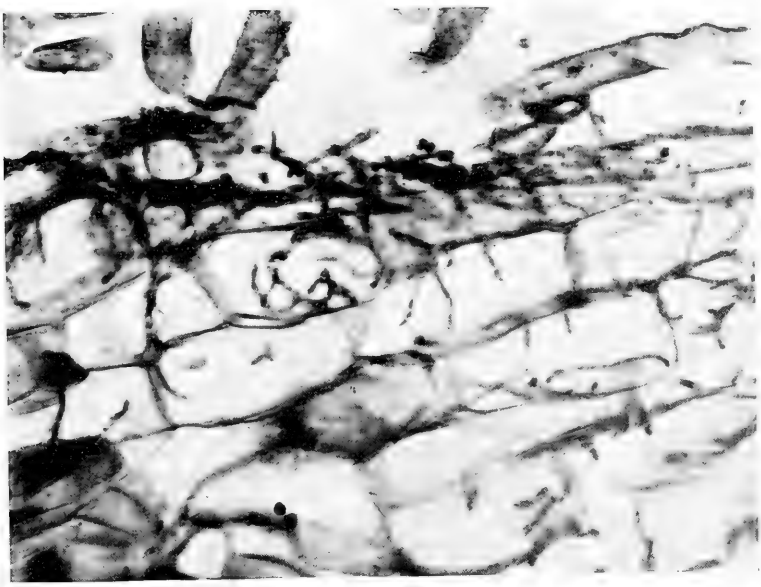
BRANDES: BANANA WILT

Plate XXXI

Fig. 1. Macroconidia and microconidia of *Fusarium cubense* stained with Bismarck brown.

Fig. 2. Radial section of young fleshy root showing method of penetration of the fungus. At the top are fragments of root hairs which do not lie in the plane of the section.

1



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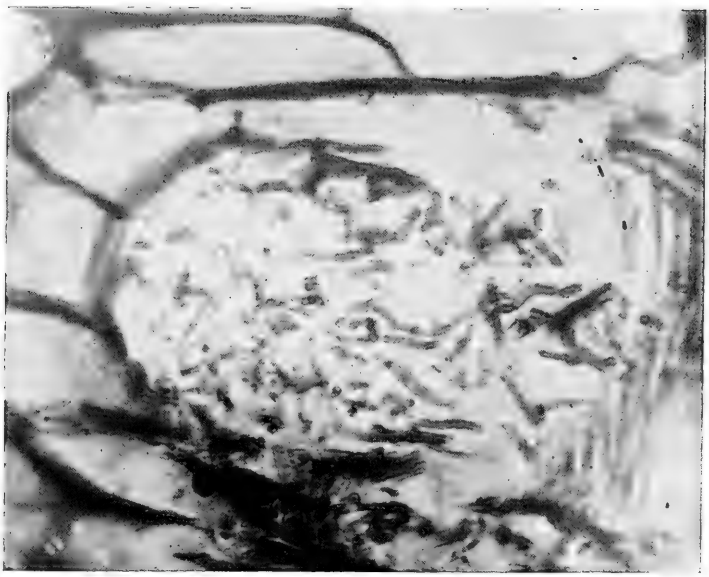
BRANDES: BANANA WILT

Plate XXXII

Fig. 1. Macroconidia germinating in distilled water. The germ tubes arise from any cell and are here long and attenuated.

Fig. 2. Transverse section of a vessel in the root. Notice luxuriant vegetative growth of the fungus.

1



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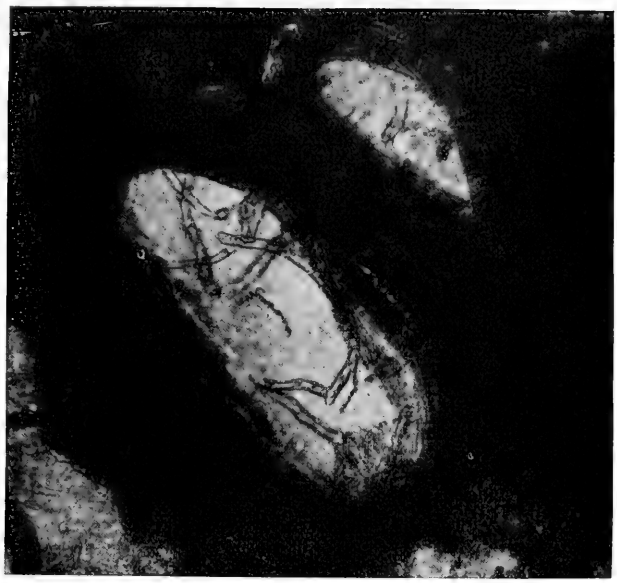
BRANDES: BANANA WILT

Plate XXXIII

Fig. 1. Transverse section of vessel in the stele of a diseased rhizome. The fungus is not so abundant here as in the vessels of the root near the point of infection.

Fig. 2. Longitudinal section of a diseased vessel in the pseudostem. Notice the scant mycelium.



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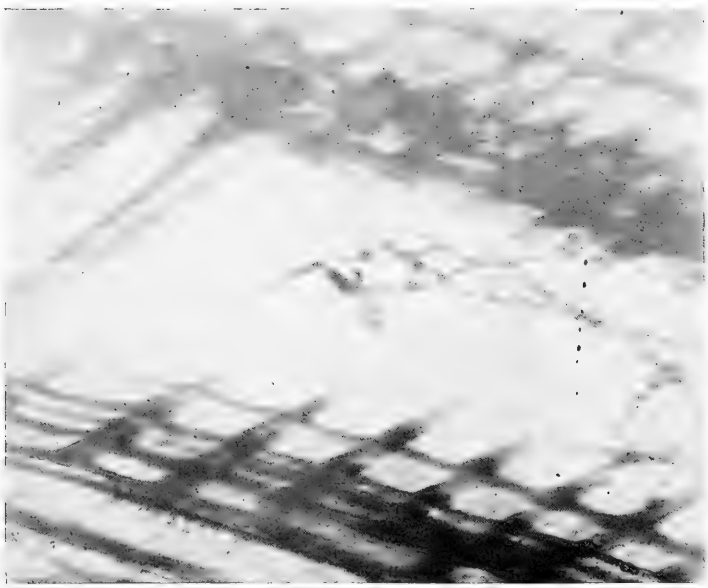


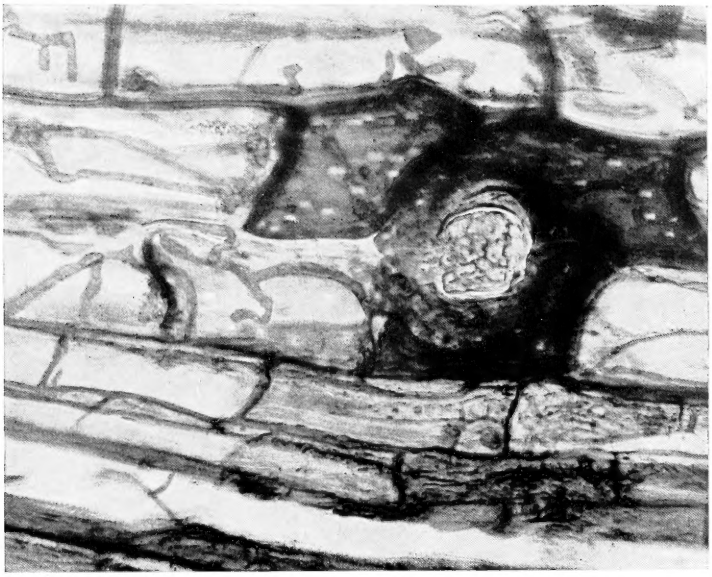
2

BRANDES: BANANA WILT

Plate XXXIV

Fig. 1. Tangential section of leaf stalk showing subepidermal cells filled with mycelium of the parasite. A substomatal cavity filled with pseudoparenchymatous tissue is present in the section.

Fig. 2. Radial section of a sporodolchium at surface of the leaf stalk.



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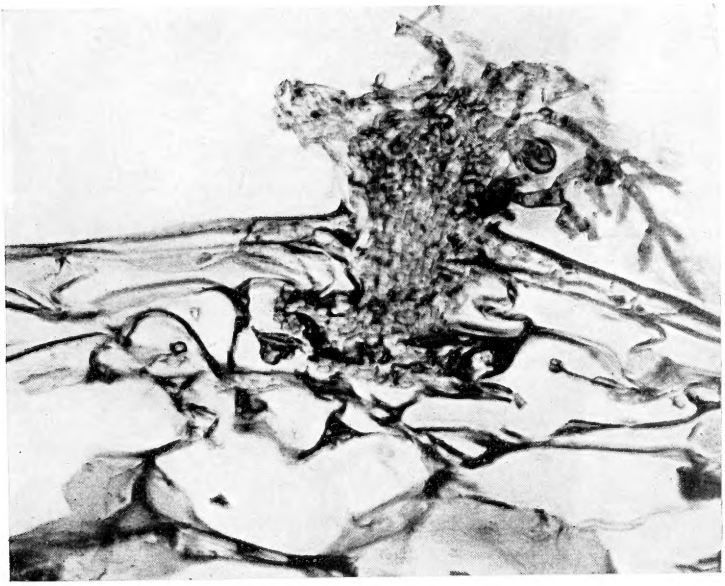

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BRANDES: BANANA WILT